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CYTO-GENETICS AND THE 'SPECIES- CONCEPT'¹

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ONE's concept of species must necessarily involve one's point of view concerning fundamental questions such as the following: Do species really exist? If so how do they originate? And how may species be recognized?

For the sake of brevity I shall assume that my audience accepts the evidence from the ever-increasing body of experience in classifying animals and plants, which certainly indicates that species do exist and that they are really natural groups of individual organisms. That is to say, with organisms in which large numbers of individuals have been thoroughly studied, the experience of many students of classification goes to show that really distinct groups of individuals have existed and do exist; that while the individuals within a group are not all alike and may differ almost endlessly and even strikingly in certain details, yet they resemble one another more than they resemble the individuals of any other group; also that the groups existing in a given time-plane usually differ from one another in numerous characteristics and are separated by definite gaps, marked by physiological and ecological divergence if not by constant morphological features, except in some cases where natural hybrids occur. Along with a certain range of variation to

¹ Read at the Fifth International Botanical Congress, Cambridge, England, August 21, 1930.

be found among the individuals composing these natural groups, there exists a relative constancy or stability for each group taken as a whole. Relative stability, combined with a definite tendency to vary, seems to be generally characteristic of these natural groups. It is this combination of the idea of the existence of natural "unit-groups" of organisms, now generally referred to as Linneons, and the idea of perpetuation of these groups over long periods of time which I am assuming to be acceptable to most biologists as a basis for the formulation of a species-concept.

Thinking of species in these terms, we may consider briefly the second question, How do species originate? My purpose in taking up this question here is not to discuss evolutionary processes in detail, but merely to take the next logical step in formulating a concept of species which shall be consistent with existing knowledge. The perpetuation of these specific natural groups is made possible by the reproduction of individuals. Sexual reproduction requires the union of two unlike gametes, and in the higher organisms these unlike gametes come from different individuals, except in self-fertilized organisms and forms which reproduce asexually or by some modified form of sexual reproduction. It is pertinent to this discussion, therefore, to inquire as to what extent the individuals of different specific groups intercross naturally and what bearing this may have on our concept of species. Many cases of intergrading forms have been found to connect up distinct groups which, except for these connecting forms, would rank as species. In some such cases experiments have proved that the connecting forms are really hybrids,² and in many others the evidence from the intergrades themselves is fairly conclusive. It is now generally accepted that many true species will hybridize and that they often produce partially fertile offspring. In plants especially hundreds of interspecific hybrids have been produced by artificial

² See especially the recent research of Marsden-Jones and Turrill on *Silene*, Kew Bull. Miscel. Infor. 1928-1929.

crossing and all degrees of fertility have been found in the first generation plants from fully fertile to fully sterile. The general tendency, however, is toward sterility and at the same time it must be remembered that a great number of such crosses have been attempted and have failed completely. If along with this evidence we consider the evidence that species, once established, tend to remain relatively stable or fairly constant over considerable periods of time, we are led to infer that, while the individuals within species may intercross freely and be highly interfertile, the individuals of different species hybridize comparatively rarely in nature and when they do cross the hybrids are more or less sterile. It must be noted in passing, however, that very recent experiments on artificial plant hybrids have proved that fully fertile, constant hybrids (amphidiploids and autosyndetic hybrids such as *Crepis artificialis*) may sometimes be produced by crossing two very distinct species. Furthermore, there is steadily accumulating evidence that in certain plant genera such as *Hieracium*, *Rosa* and *Betula*, interspecific hybridization is rather common and sometimes gives rise to very numerous hybrid forms which perpetuate themselves by some form of apomixis. Many such forms have been described as species and their hybrid nature has been discovered later by cytogenetic investigations. Natural hybridizing of species, therefore, may be recognized as one method of origin of species without invalidating our concept of species. But from existing evidence it is clear that interspecific hybridization can hardly be thought of as the only process making evolution possible.

Further evidence bearing on this question of how species originate has come from intensive studies of plant and animal groups which have led to the publication of monographic treatises on classification. Such studies have shown the existence within species of sub-specific groups, often occupying different geographic areas and differing more from one another in morphology or interfertility or both than do the individuals

within the subgroups. The very existence of such subspecies or varieties is generally looked upon as good evidence that Darwin was correct in his fundamental idea that the existing differences between many species are the result of gradual accumulation of such smaller differences as distinguish the subgroups within a species. At this point it is unnecessary to discuss the genetic processes which make possible this differentiation within species. These will be mentioned when considering the bearing of the evidence from cyto-genetics on the species-concept.

Existing evidence seems to indicate that both the Darwinian and the Lotsyan hypotheses of species origin are correct in part; *i.e.*, many of our present species must have arisen through gradual differentiation, aided by isolation, within preexisting species and, on the other hand, some of our present species are probably the result of natural hybridization followed by the establishment of one or more new constant forms combining some of the characteristics of the parent species. Apparently there is nothing in either of these hypotheses as to origin of species which is inconsistent with our concept of species as thus far developed. The essential ideas in this concept may be summarized as follows:

(1) Common structural characteristics which unite certain individual organisms into one group, the species.

(2) Characteristic features which distinguish such groups from one another.

(3) Relative stability combined with more or less variability within the group.

(4) Common descent of all the individuals of the group from one or more preexisting species.

(5) Free intercrossing and high (but not necessarily complete) interfertility among the individuals of the group.

(6) Absence of free intercrossing and usually low fertility if not complete sterility in hybrids between different species (although highly fertile and constant new forms may sometimes arise in this way).

(7) The frequent occurrence of subspecific groups, often occupying different geographic areas which differ more from one another in structure or interfertility or both than do the individuals composing each subgroup.

The third question raised as pertinent to our problem of formulating a species-concept was, How may species be recognized?

The recognition of types and the preparation of diagnoses or detailed descriptions of types were essential methods of the museum or herbarium worker who frequently recognized as "species" whatever new individuals failed to correspond sufficiently in his judgment to any species already recognized. Many of the so-called species which were named and classified by this method turned out later to be merely forms, varieties or subspecies of the same or some other species. When the partial failure of older methods became more apparent other aids were invoked. Some writers emphasized *distinctness* as a valuable criterion, but well-marked varieties or subspecies are very distinct. Others, notably Darwin, emphasized *constancy* or the ability to breed true. This is of much greater value, especially if used in the general sense of constancy in the more important distinguishing characters of the species. We might combine the two and speak of constant distinctness between true species. But within most species there is variation from generation to generation and never strict constancy.

The growing dissatisfaction with former methods of classification led to various suggestions and proposals, some of them being so radical as to advocate abolition of the term species from biology. Another radical proposal was that of Lotsy who advocated gametic purity as the sole test of the validity of species. But if this criterion were adopted the number of species would become inconceivably large and the natural unit-groups, the Linneons of Lotsy, would still exist and require classification. Other scholars, notably Poulton, appealed to taxonomists to name new species with reservations and with a deter-

mination to seek for further evidence which might or might not warrant their permanent recognition.

More recent trends in taxonomic work have placed emphasis on field study, including geographic distribution and ecological relations, the use of statistical methods in describing species, the development of methods of experimental ecology and finally the application of the results of investigations in genetics and cytology to problems of classification. Each of these developments contributed something to the species-concept. Supplementing museum work with field study brought to light many significant facts concerning the geographic areas occupied by related species, with resulting inferences as to their comparative ages and phylogenetic relations, and this in turn had a bearing on the principle of common descent of the individuals within a species. The use of statistical methods in the study of variation within a species has been of value in establishing the existence of subspecies in many plants. The work on experimental ecology has thrown considerable light on the nature of the variations found among the individuals of which species are composed. Thus Turesson, in Sweden, has shown that many species of plants which are wide-spread and occupy different habitats are composed of different hereditary races which he calls ecotypes because they are distinct forms which through selection have become adapted to different sets of ecological conditions. This is further evidence of such differentiation within species as may lead eventually to the origin of new species. Again, Hall, in California, by his transplant experiments has been able to confirm Turesson's findings and has also shown that certain supposed "species" are merely transient forms induced by peculiar environmental conditions. These modified forms do not retain their original distinguishing features when transplanted to a common testing garden under uniform conditions, which shows that their features are temporary variations induced by the environment. This in turn necessitates the revision of our descriptions of the particular species in question.

In brief we find that all these more refined methods of study have yielded results which strengthen our conviction as to the validity of the seven basic ideas with which we have attempted to formulate a concept of species.

The contributions of genetics and cytology to the species problem will be considered somewhat more in detail. A concept of species, to be consistent with the facts of evolution, must harmonize the apparently paradoxical phenomena of constancy of species and variety of forms within species. The integration of these diverse tendencies is adequately provided for in the known material basis of heredity and genetic variation.

I. THE BASIS OF HEREDITY

There is a distinctive, relatively stable, hereditary complex for each species. This relatively stable heritage consists of many different primary elements, the genes, which are carried in a practically constant group of chromosomes, peculiar to the species. This constant chromosome group consists of n pairs of chromosomes where n = the number of chromosomes in the gametes of the species. Each chromosome carries a linear series of genes and in each pair of chromosomes the genes are homologous and normally occupy identical loci. With reference to the nature of the genes they carry, each pair of chromosomes differs from every other pair but the genic differences between the chromosomes are not necessarily accompanied by visible differences in the chromosomes of a species. In many genera of plants the chromosomes are so small or so similar in size and shape that no constant morphological difference between them can be observed. In many such genera, however, number of chromosomes has proved a very useful criterion, an outstanding example being J. Clausen's work on the genus *Viola*. On the other hand, in some groups of organisms the number, size and shape of the chromosomes are very useful as additional criteria for the recognition of species and the determination of relationship between species. These criteria, however, should

always be used in connection with all other available criteria. In accepting the chromosomes as the chief material basis of heredity, one should not overlook the cytoplasm and the important rôle it plays in development of the individual; and certain actually demonstrated cases of cytoplasmic inheritance must be admitted. But, in view of available evidence, the chromosomes must be recognized as the basis of Mendelian heredity, and it appears that heredity is mostly Mendelian in all plants above the thallophytes if not in them.

II. THE GENETICS OF VARIATION

The differences found among the individuals within species have been rather extensively analyzed by cytologic and genetic methods. In the wild state the individuals in a population of any sexually reproduced animal or plant exhibit both structural and functional differences from one another. Many of these variations are merely the somatic effects of environmental differences and are not inherited, but some of these differences among the individuals in wild populations are found to be inherited. Furthermore the study of various populations of the same species, especially populations from different geographic regions, reveals the existence of still more inherited differences. During the decade just closing a vast amount of work has been done in analyzing the genetics of these intraspecific hereditary differences. As a result of such investigation we are now in position to generalize to some extent.

Intraspecific differences which are inherited fall into several different categories according to the nature of the germinal changes involved. The most important of these categories, considered with reference to the origin of species, are three in number as follows:

1. *Genic variation*: Genic differences in an otherwise normal chromosome complex condition both qualitative and quantitative differences among the individuals of a species. In a single species of fruit fly hundreds of such varietal differences are known and as a rule each pair of

differences involves only one pair of genes. Similar simple genic differences are known in hundreds of other animals and plants. Also a great many varietal differences are known, especially quantitative differences, which involve two or more pairs of genes simultaneously. These genic differences arise *de novo* as gene mutations which can be produced experimentally. The existence of these numerous genic differences makes possible the occurrence of innumerable natural subspecific forms resulting from various combinations of genes in cross-fertilized species, and in self-fertilized species natural populations are more or less heterogeneous due to gene mutations and occasional crossing. That these gene mutations function as the basic process in the origin of species has been advanced by Morgan, Muller and others. The existence of highly polymorphic species in which the numerous subspecific forms differ in respect to individual genes but which exhibit a uniform chromosome complex certainly indicates that gene mutations make possible intraspecific differentiation. But genic variations, whether due to chemical changes in individual genes or occasional loss or addition of genes, are inadequate to account for the differences in chromosome number and morphology which exist among the species of many genera to say nothing of similar differences between genera, and between the larger natural groups.

2. *Chromosomal variation*: Rearrangements of parts of chromosomes have been found in nature. A fragment of a chromosome has been added to the normal chromosome complex in certain species of *Crepis*, *Nicotiana* and other genera. In maize a chromosome of one variety may be represented by two smaller chromosomes in another variety. Sometimes such a fragment becomes attached to one of the other chromosomes. Such rearrangements of parts of chromosomes sometimes affect intraspecific variation; they also seem the most likely cause of known cases of differences in arrangement of corresponding genes in closely related species of *Drosophila*. In this connection it should be noted that

chromosomal variations including both translocation and deletion of parts of chromosomes can be caused experimentally; also that studies of chromosomes in relation to phylogeny in several plant genera, notably in *Muscari*, in some of the *Polygonaceae*, in *Vicia* and more recently in *Crepis* have produced considerable evidence that the evolution of these genera has been accompanied by gradual transformation in chromosome size, and has led to the conception that changes in chromosome number may have arisen in this way. On the other hand, the well-known deleterious effects on the organism of gross unbalance in the chromosome complex makes it necessary that such hypothetical transformation of the chromosomes proceed very gradually, by minute subtractions or additions in an order of magnitude corresponding to very small groups of genes, or individual genes or even parts of genes. Whether such gradual transformation of chromosomes is actually going on in nature and whether this process is more or less fundamental than gene mutation in the general method of evolution remains to be discovered.

3. *Polyploidy*, or balanced increase of the entire chromosome complex, sometimes produces very distinct forms of existing species. The most commonly occurring polyploid forms are triploids and tetraploids. Triploids are only slightly fertile, yet even with low fertility they may sometimes serve as the starting point in the origin of a series of polyploid forms as has been shown by M. Navashin in his work on triploids in *Crepis capillaris*.⁸ Tetraploids are self-fertile and usually more vigorous than the diploid ancestor. Furthermore they frequently do not cross readily with the diploid form and when they do the progeny are mostly triploids which are sterile. All this favors perpetuation of tetraploid forms. Thus in polyploidy we find a process which makes possible the fairly rapid rise of a new species with $4n$ chromosomes from a $2n$ progenitor. In view of existing

⁸ M. Navashin, "Studies on Polyploidy I. Cytological Investigations on Triploidy in *Crepis*," Univ. Calif. Publ. Agr. Sci. 2: 377-400. 1929.

evidence, however, polyploidy, like interspecific hybridization, in general seems to be of secondary importance in the origin of species. The primary processes appear to be gene mutation and chromosome transformation. These ideas are not in conflict, necessarily, with Lotsy's suggestion that hybridization may have been of importance in the origin of certain phyla.

Polyploidy and other variations in chromosome number have made necessary some modification of the old idea that each species has just one specific number of chromosomes. Thus in all the true polyploid forms derived from the same diploid species the same specific chromosome complex occurs throughout, although intra-specific variation may be considerably increased. And if, morphologically, such a group of forms may still be considered one species, then the mere fact of such variation in chromosome number need not prevent such taxonomic treatment. Again, a few cases of variable chromosome numbers within species are known, for example, *Viola canina*⁴ and *Crepis syriaca*.⁵ Morphologically each of these groups must be considered as one species. They must be looked upon as special cases or exceptions to the general rule that all the members of a species have the same chromosome number.

We may now return to the species-concept previously formulated and amplify each of the seven essential ideas in the light of evidence from cytology and genetics. These may then be stated somewhat as follows:

1. Common structural characteristics which unite certain individual organisms into one group, and a common genetic basis for the group represented by a specific chromosome complex or *genom*.
2. Characteristic features which distinguish such groups from one another, one of these features frequently being the chromosome garniture (*genom*).

⁴ J. Clausen, unpublished data.

⁵ L. Hollingshead and E. B. Babcock, "Chromosomes and Phylogeny in *Crepis*," Univ. Cal. Publ. Agr. Sci. 6: 1-53. 1930.

3. Relative stability combined with more or less variability within the group. This stability is made possible by a high degree of regularity in chromosome distribution from cell to cell and from parent to offspring, while inherited variations arise from occasional changes in genes and chromosomes.

4. Common descent of all individuals of the group from one or more preexisting species is made possible by the known mechanism of heredity and genetic variation.

5. Syngamy or free intercrossing and high interfertility among the individuals of the group are just what would be expected in organisms in which a large majority of the genes in all the chromosomes are homologous.

6. Absence of free intercrossing and usually low fertility if not complete sterility in hybrids between different species (with a few exceptions including amphidiploids) are logical results of the accumulation of genic and chromosomal differences between diverging groups of individuals within a species.

7. The existence of subspecific groups often occupying different geographic areas, which differ more from one another in structure and interfertility or both than do the individuals composing each subgroup, but these subspecific groups sometimes still connected with one another by intergrading forms. This is the necessary result of genetic variability within the species plus the influence of environmental variability, isolation and natural selection. It is a stage in one process of species formation.

Thus it appears that the evidence from cyto-genetic research on intraspecific and interspecific variation can be combined with the basic ideas in a species-concept so as to add considerably to the definiteness of the concept. Furthermore, cytology has brought forth additional useful criteria for distinguishing species, especially the number and morphology of the chromosomes. And finally cyto-genetic investigations are coming into general use for testing the genetic nature of plants suspected of being hybrids and as a helpful method of determining the degree of relationship between species of the same genus.

It will be noted that nowhere in this paper has a *definition* of the term species been proposed. Darwin considered the term to be indefinable and yet it is obvious that he made continual use of a very definite concept of species. It has long seemed to the writer that it should be worth while to attempt to formulate such a concept in the light of present-day knowledge and it is hoped that the foregoing propositions will prove useful as a temporary working basis; no doubt these ideas will need to be extended or modified. Furthermore, it should be noted that the phylogenetic concept of species has not been considered here inasmuch as this aspect of the problem has been discussed in earlier publications.^{6, 7}

In this connection should be noted the excellent and timely contribution of Vavilov, represented among the abstracts of papers to be read at the Fifth International Botanical Congress. Although this paper was not delivered, the abstract presumably will appear in the published proceedings of the congress under the title: "The Linnaean species as a system." That Vavilov is dealing with essentially the same concept of species, and in the same relation to evolution, as the present writer, is indicated by the following paragraphs quoted from his abstract.

Thus, a *Linnaean species* is, according to our conception, a *separate morpho-physiological system connected in its genesis with a definite environment and area.*

In opposition to the rather common notion as to the relativeness of species and the conventional character of their understanding, the great number of concrete facts encountered in studying the cultivated plants and their wild relatives, has induced us to recognize the Linnaean species as actual complexes, actual systems, which exist in nature and represent important definite links in the evolutionary chain, the knowledge of which is very helpful in mastering the multifariousness of the organized world.

⁶ H. M. Hall and F. C. Clements, "The Phylogenetic Method in Taxonomy," Carnegie Institution Publ. 326: 3-30. 1923.

⁷ L. Hollingshead and E. B. Babcock, "Chromosomes and Phylogeny in *Crepis*," Univ. Cal. Publ. Agr. Sci. 6: 1-53. 1930.

The concrete material shows that the so-called "Linnaean species" may be very different in regard to their contents. Being more or less separate systems, they manifest themselves in a *different compass*. The analysis of a great number of Linnaean species by means of the method of differential systematics and differential geography, as well as by the modern methods of genetics and cytology, reveals the great diversity of the species. Thus, there are sometimes bulky systems belonging to one Linnaean species, which might be expediently subdivided into categories or subspecies. Sometimes, on the contrary, a Linnaean species represents a very limited and comparatively small system.

The conception of a species must not be obliterated. Through our knowledge of the multifariousness shown by cultivated plants and by their relatives, of plant groups where the chaos of diversity seems to be boundless, we have come to conceive of a species as a regular system which may embrace categories of different compass. The species, as a conception, is necessary not only for the sake of convenience, but for a real comprehension of the essence of the evolution process. The process of evolution may proceed uninterrupted on its fundamental lines, but it has nodes in its chain, which are the species representing systems of hereditary forms.

In conclusion let me emphasize the importance of using all possible criteria for recognition of species in pursuing the work of classification, especially in preparation of taxonomic monographs. General works on floras and faunas are indispensable and in these classification will doubtless continue to be based primarily and largely on morphological resemblances and differences together with data on geographic distribution. But in the detailed study of restricted groups of organisms as many as possible of the essential features of the species-concept should be tested. This requires cytologic and genetic study which in turn calls for living material; it also calls for field observations including ecological relations, barriers, species boundaries, overlapping and occurrence of hybrid forms. Although difficult, time consuming and expensive, such comprehensive research offers a really worth-while opportunity for a broad and intensive attack on fundamental biological problems.

CYTOLOGICAL EVIDENCE AS TO THE STATUS OF DROSOPHILA MELANOGASTER

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IN 1925 the author published mainly in collaboration with Professor G. C. Hicks, now of the University of Buffalo, a number of articles dealing with the cytological conditions present in the reduction divisions of *D. melanogaster*, which in the United States and to a large degree in the world beyond is the *corpus vile* of genetical teaching. These were three in number and appeared in *Science*, the AMERICAN NATURALIST and in *Genetica*. The conclusions stated in these various articles were that the cytology of this interesting and most variable species clearly showed it to be of heterozygous or hybrid origin, since its reduction divisions presented an exact parallel to those found in interspecific and intergeneric hybrids. The reactions of geneticists in this country and abroad have had in the interval time to develop. The general attitude of American genetical workers has been either that of silence or contempt. The Europeans as is generally the case have shown themselves to be not only more open-minded but also of broader outlook than most of their American colleagues. It is unfortunately assumed by many American geneticists that the cytology of *D. melanogaster* is of little importance compared with its genetical behavior. This situation is all the more remarkable because American geneticists in general hold much more confidently to the chromosome theory of inheritance than do their European colleagues. Frequently, too, those who are favorably disposed towards cytological investigations assume that the results obtained in the author's laboratory are erroneous or the result of the use of inferior technique. This attitude is very frequently present in personal discussions but is

more rarely manifested in publication, where naturally more prudence in statement must be observed.

The first important criticism of our work on *Drosophila melanogaster* is that published in 1928 by Belar in Lieferung 5 of the "Handbuch der Vererbungswissenschaft" edited by Baur and Hartman (Berlin, Gebrueder Borntraeger, 1928). The author in this case supports his criticisms by photomicrographs, which are supposed to show a normal behavior of the chromosomes in the reduction divisions. All the photomicrographs of the divisions in the primary spermatocyte indicate a clear lagging of chromosomes, precisely as first described by us in 1925. The illustration of the second division, however, does show a normal behavior of the chromosomes, but that is frequently found to be the case in known hybrids as well as in numerous extremely variable species of plants, which are with good reason suspected to be of hybrid origin. Belar attacks our technical procedure, suggesting that our faulty technique has caused us to confuse mitochondria (chondriosomata) with chromosomes. A very frequent criticism of our work in fact has been directed at our supposed confusion of chromosomes with other structures of the cell, not only mitochondria but also even nucleoli or plasmosomata. These criticisms will be dealt with later in connection with our own observations.

In an important communication published in an English résumé in the seventh volume of the publications of the Genetical Bureau of the University of Leningrad, Zuitin asserts the chromosomal character of the lagging bodies present particularly in the first reduction mitoses of *D. melanogaster* and in this respect corroborates the description published by us in 1925. He further emphasizes the abnormal character of the reduction division of *D. melanogaster* and interprets it as an indication of the hybrid interspecific origin of this species.

More recently Miss League has published a summary of her observations on the meiotic phenomena of this

much discussed species¹ in which she concedes the abnormal character of the reduction divisions in the species but interprets the lagging structures not as chromosomes but as plasmosomata.

Later the present author discussed and criticized Miss League's observations² as well as a less important communication by Huettner.³ It was pointed out that it was impossible to regard the lagging bodies which are particularly clearly present in the first reduction division as plasmosomata. Huettner in his article cites an article published recently in *La Cellule* in opposition to our views but has no evidence based on his own observations of the male mitoses to offer. He particularly commends, however, the use of Feulgen's reaction for the detection of chromatin as destined to settle the argument in favor of the point of view of American Drosophilists. The use of Feulgen's reagent, however, has not proved favorable to the view of the critics of the chromosomal character of the lagging bodies in the reduction divisions of *D. melanogaster*, for these structures unmistakably give the reactions of chromatin and consequently can not be regarded as related either to chondriosomata or plasmosomata. These important observations published by Woskresensky and Scheremetjewa⁴ seem to set at rest the chromosomal nature of the lagging bodies of which such fantastic explanations have been recently furnished by recent authors, apparently unfamiliar with the cytological peculiarities of hybrids. The present author has likewise treated a large amount of material of the species under discussion with Feulgen's reagent and is able to confirm absolutely the observations of the two authors just cited. Not only do these authors find the abnormal lagging bodies present in the mitoses of the reduction

¹ *Science*, 71: 99, January 24, 1930.

² *Science*, 71: 315-17, March 21, 1930.

³ *Science*, 71: 241, February 28, 1930.

⁴ "Die Spermiogenese bei *Drosophila melanogaster* Meig.," *Zeitschrift fuer Zellforschung u. mikroskopische Anatomie*, Bd. 10, Heft 2, 15 January, 1930.

phase of the species under discussion to be chromosomal but they also note the same multiplication of number in the chromosomal equipment in the reduction division earlier described and figured by Dr. Hicks and the present author. This is a further feature of known or justly suspected interspecific hybrids. Its presence has been the special object of ridicule of purely experimental



FIG 1

biologists. It seems clear that the experimental methods alone in the biological sciences can lead to no permanently important result. The comparative and morphological data are obviously necessary to complete the picture.

It is now possible to describe our own mature observations on the meiotic phenomena of *D. melanogaster*. It will serve a useful purpose in this connection to refer first to the somatic and reproductive divisions in that

classic object of cytological investigation *Allium cepa*, the common onion. Fig. 1 is a reproduction of a highly magnified photomicrograph of the nuclear divisions of this species as seen in the tips of the roots. On the left of the figure are seen cells in stages of transition from the resting conditions to that of karyomitosis. The second cell from the top on the left shows a typical resting nucleus. In the cell above it and the two below the nucleolus is passing into the active stage and the nuclear reticulum is giving place to a definite thread-like and much convoluted spireme. In all the stages described

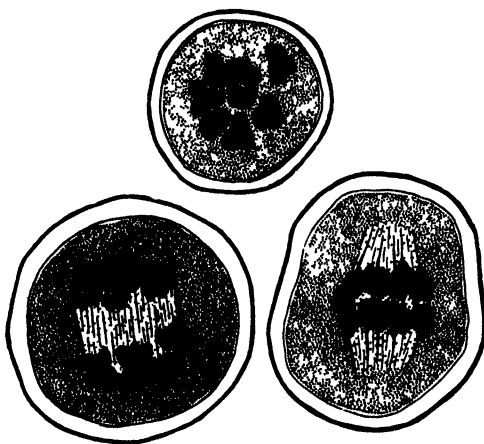


FIG. 2

and shown on the left of the photogram, in addition to the nuclear filament or spireme, there is present a darkly staining body (sometimes two or more), the nucleolus, which is sometimes surrounded by a lighter halo. To the right of the photogram are shown three well-recognized stages in nuclear division, namely, the metaphase, anaphase and telophase of mitosis. In these only chromosomes or their derivative the spireme can be distinguished. The nucleoli so distinctive of the resting stage and the earlier phases of preparation for division are conspicuous by their complete absence. In Fig. 2 are shown the reduction phenomena of *Allium cepa*. In the

item at the top of the figure the metaphase of mitosis is shown in polar view, exhibiting eight chromosomes, half the number shown in the somatic or sporophytic divisions. To the right below appears the metaphase in lateral view, showing clearly in this aspect the synapctic mates or bivalent chromosomes, which in this phase appear to be related end to end (telosynapctic mates). To the left appears the anaphase of the meiotic first division. Here again we see nothing whatever of lagging bodies of any description, whether plasmosomes or nucleoli. The illustrations, photographic and graphological, sporophytic and gametophytic, show in fact no indication of lagging bodies either in the metaphase or anaphase. This is the general condition in clearly defined species and may consequently be described as the normal condition for normal species.

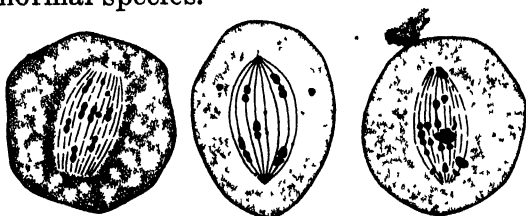


FIG. 3

We may now turn with advantage to the meiotic conditions in known hybrids. In Fig. 3 is shown on either side the metaphase of interspecific hybrids. On the left is represented the meiotic division of a hybrid hazel (*Corylus*) and on the right that of a hybrid violet, borrowed from the recent publications of Woodworth on the *Betulaceae* and of Clausen on the genus *Viola*. In the center appears the reduction division of a triploid *D. melanogaster* in which appear six pairs of chromosomes constituting telosynapctic mates. The close general resemblance of this figure to the meiotic divisions of the two known hybrids appearing on either side is obvious.

In Fig. 4 appear the phenomena of the anaphase of meiosis in the species under discussion. On the left is

shown the anaphase as represented in our article published in 1925. Obviously in this phase as in the preceding metaphase there is marked lagging of bodies resembling in every respect chromosomes. The next item to the right represents one of Zuitin's figures, showing a somewhat later stage of the anaphase. The lagging and extrusion of what is apparently chromosomal material is as marked here as in the illustration immediately to the left. In both figures there is a perinuclear halo of mitochondrial material, which on account of the nature of the preservative used is indistinctly seen. To the extreme right parallel figures above from the author's earlier description and below a somewhat later stage of

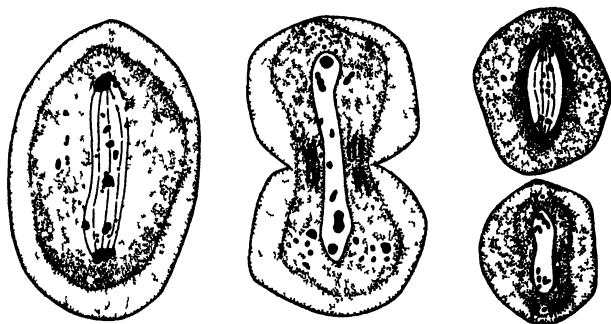


FIG. 4

the anaphase of the second division from Zuitin's article already cited. The lagging of bodies interpreted by both the present author and Zuitin as chromosomes is clearly seen.

It has been emphatically asserted by several American authors that the lagging structures represented in the two last figures are either the result of imperfect fixation or correspond to other structures of the cell than chromosomes. The accepted reagent at the present time put forward by the biochemists for the diagnosis of chromatin is that of Feulgen, which depends on hydrolysis with dilute hydrochloric acid, followed by treatment with basic Fuchsin and sulphurous acid. In Fig. 5 is shown the topographic distribution of the chromosomes in the first

meiotic division as demonstrated by this reaction. This figure is copied from the article by Woskressensky and Scheremetjewa cited above. The present author by the use of the method of Feulgen has reached substantially the same results as these authors and consequently confirms their conclusions. It has appeared better, however, to reproduce their figures rather than to present original illustrations. The two items at the top illustrate the metaphase with lagging bivalent chromosomes. The two drawings below visualize the anaphase of the first meiotic division, and furnish confirmation of the



FIG 5

present author's observation of strongly lagging and multiplied chromosomes in this phase. Surrounding the area occupied by the spindle-shaped divisional figure are the mitochondrial or chondriosomal perinuclear zones.

The writer has been severely although not very logically criticized for employing Carnoy's alcohol-chloroform-acetic acid fluid for preservation. It is generally admitted by competent cytologists that this reagent is one of the best for the preservation of the chromosomal structures, which are the most important elements in the present discussion. It is true that this reagent does not

preserve so well the cytoplasmic structures. On the other hand, those reagents which have a favorable action on the cytoplasm are less successful in the case of the nucleus. It has been thought wise as a consequence to compare the action of the two types of reagent in photomicrographic illustration. Fig. 6 at the top shows three primary spermatocytes of *D. melanogaster* preserved with Carnoy's fluid. The figure is clearest in the cell on the left, where the chromosomes are scattered longi-



FIG. 6

tudinally over the spindle as univalents. A number of these have already reached the poles and constitute a dense mass in which the identity of the chromosomes is largely lost. The cell in the center is obliquely sectioned and does not as a consequence show clearly. On the extreme right of the upper item appears another anaphase, less clear than that on the left. Below on the right is seen the anaphase of the left side of the upper item, more highly magnified. On account of the twisted form and small size of the sex organs in *D. melanogaster* it is difficult to get axial sections of the cells. This situation together with the small size of the spermatocytes makes

clear photomicrographs difficult to obtain. On account of the difficulties just described, the metaphase of the primary spermatocyte is shown at different foci in the two items below and on the left of the figure. In the extreme left is shown one end of the nuclear figure in focus. The next item shows the other end in sharp focus. Only four of the telosynaptic mates appear with some degree of clearness. Two others present in this preparation are not shown, including the small rounded pair, so familiar in the figures of the somatic cells of *D. melanogaster*. In Fig. 3 the middle item shows this cell complete with the

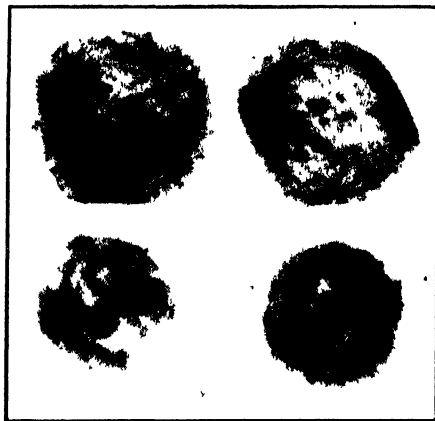


FIG. 7

six pairs of chromosomes, corresponding to the triploid condition described by Morgan in his monograph of the species, published in *Bibliographia Genetica* in 1925.

The upper item of Fig. 7 shows the secondary spermatocytes of the species in the anaphase. The lagging of the chromosomes is very obvious (preservation with Carnoy's solution). In the lower photomicrograph appear two cells in the metaphase, preserved with Champy's fluid consisting of chromic acid, bichromate of potash and osmic acid. This is one of the reagents highly prized by those whose special interest lies in the structures of the cytoplasm. To the left appears a somewhat normal

metaphase, while on the right the metaphase is characterized by the strong lagging of univalent chromosomes. It is easy to parallel such contrasting figures in the meiotic phenomena of known hybrids between species.

In Fig. 8 are shown views at different foci of secondary spermatocytes in the later stages of division preserved with Benda's fluid, consisting of chromic acid, osmic acid and a mere trace of acetic acid. The preservation of the cytoplasmic structures in this photomicrograph is particularly good. The mitochondria are arranged in two groups on either side of the equator of the dividing cell. Although the division is far advanced the chromosomes can still be seen lagging in their procession to the poles.



FIG. 8

It seems clear from the various data introduced in the present article that the meiotic phenomena of *D. melanogaster* are very far from normal and to any one who has a reasonably complete acquaintance with the cytology of hybrids, particularly of hybrid plants, concerning which our knowledge is less incomplete, present an unmistakable indication of hybrid contamination. The imprudent assertions made from time to time, especially in the less responsible occasions of private intercourse, as contrasted with scientific publications, as to the complete normality of this species, appear to possess no real foundation in fact. Not only are lagging bodies exactly comparable to

those present in known hybrids found in the meiotic mitoses of this species but these can be demonstrated by the best approved technique of biochemistry to be of the nature of chromatin. The strong resemblance of the maturation divisions in the male gonads in *D. melanogaster* to those of the male meiotic mitoses of known hybrids can not remain without significance to those biologists whose interests lie in the general point of view which appears to be most truly scientific. Further, the abnormalities presented by the reduction divisions of this protean species offer the best possible explanation of its extreme variability. This explanation is moreover quite in line with our ever-increasing knowledge of the cytological phenomena presented by the extremely variable species of large genera, long ago emphasized by Darwin in the "Origin of Species."

SUMMARY

1. The use of various methods of preservation, comprising those which conserve with the greatest fidelity the nuclear and cytoplasmic structure, respectively, makes it clear that the reduction phenomena of *D. melanogaster* are quite abnormal.

2. The abnormalities present are those of known hybrids and of the often extremely variable species of large genera.

3. Assertions that the lagging structures in this species are not chromatin are disproved by the fact that they clearly give the reactions of chromatin with Feulgen's reagent.

4. Even if they were not chromatin they would constitute a thoroughly abnormal condition of which there is no reasonable explanation other than that of previous interspecific hybridization.

DO EARTHWORMS GROW BY ADDING SEGMENTS?

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INTRODUCTION

It has been a common assertion that one of the factors contributing to the growth in length of earthworms is the laying down of new segments. The process of segmentation is said to continue beyond the embryological stage of development in the cocoon. Pearl and Fuller² are

¹ This investigation was undertaken and completed at Ohio State University.

² Pearl, R., and Fuller, W. N., "Variation and Correlation in the Earthworm," *Biometrika*, 1905-06, 4, 213-229.

Up to a certain age at least, *Lumbricus* adds somites at the posterior end of the body with growth. It is clear . . . that the great extent of the total range in the variation in this character arises from the presence of a comparatively small number of individuals with few somites which unduly extend the lower end of the range. It seems reasonably certain from what we know of the method of growth in the earthworm that if this sample of worms could have been allowed to go on growing the range of variation in total number of somites would have steadily decreased with lapse of time.

The fact that there is no apparent tendency towards extreme extension of the range at the upper end indicates that the bulk of the worms included in the sample have either completed the process of adding somites or at least that the process is going on very slowly if at all. . . .

The conclusion, that the earthworm is more variable in length than in number of somites, may seem at first sight paradoxical. But it is really no more so than to conclude, what is very obviously the fact, that man is more variable in respect to sitting height than in respect to number of vertebrae. It simply indicates that in the earthworm we have two kinds of growth occurring together: one the addition of somites, the other increase in size of the individual somites. That these two kinds of growth are acting together will be clear when the correlation surface between total number of somites and length of body is examined (p. 218).

It is seen at once by mere inspection that the relationship between the two characters is not the same in all parts of the correlation table. Thus the worms in the two lowest length classes (i.e., from 10 to 15 cm in length)

even of the opinion that the addition of segments proceeds after a worm is sexually mature, although the rate of addition progressively diminishes. The place of origin of new segments is in dispute: Pearl and Fuller state that the segments are laid down at the posterior end, Morgan³ that they are intercalated between the posterior segment and that adjoining.

The data confirming the contention that increase in length of an earthworm is partially dependent upon an increase in segments seem to have been available to these writers, although references are not cited. In so far as this conception depends for confirmation upon the statistical analysis of measurements of mature worms rather than upon the observation of changes which occur in species characteristics during developmental history one is probably justified in questioning its validity.

THE PROBLEM

The problem arose out of a chance observation of Dr. W. M. Barrows and one of the writers that the number of segments found in each of two worms (probably *Helodrilus foetidus*) just emerging from cocoons was essentially the same as that noted in mature specimens of the

are not, in general, individuals having few somites, but instead these arrays center well towards the right end of the table. The five worms having the smallest number of somites all fall in or above the third length class (p. 219).

³ Morgan, T. H., "Regeneration," 1901, The Macmillan Co.

If we examine the method of regeneration from the posterior end of a piece of an earthworm, we find that when several or many posterior segments have been removed a new part comes back, composed at first of a very few segments. The terminal segment contains the new posterior opening of the digestive tract. New segments are now formed just in front of the terminal segment, the youngest being the one next to the end-segment. The process continues until the full complement of segments is made up. . . . Comparing these results with those described above for the anterior end, we find, in both cases, that only a few segments are at first formed, but in the posterior regeneration new segments are intercalated near the posterior end. This process of intercalation is the characteristic way in which many annelids add new segments to the posterior end, as they grow larger and longer (p. 9).

(The experiments were made upon *Allolobophora foetida*, referred to in this paper as *Helodrilus foetidus*.)

species. This observation raised the question whether the process of segmentation continues beyond the cocoon stage, or whether it terminates at some stage of embryological development prior to emergence from the cocoon.

The problem, then, is twofold:

(1) To determine whether or not the process of segmentation terminates in the cocoon stage.

(2) To obtain data showing some of the species characteristics of mature members of the species *Helodrilus foetidus* in terms of means and their probable errors, measures of dispersion and correlations between the various physical traits.

METHODS

Three stages of development were selected for investigation:

(1) Worms just emerging from cocoons;

(2) Worms having no clitella (collected from the compost heap);

(3) Worms selected on the basis of clitella—these being considered indicative of sexual maturity.

Cocoons were collected from a compost heap upon the university farm in the spring of 1927, the dates ranging from April 6 to May 2. These were washed and placed upon moist filter-paper in shallow covered glass dishes. It was made a matter of routine procedure to make a daily count of the segments of the newly hatched worms and to obtain measurements of their length. This is a difficult problem because the worms are very small, and when viewed under a microscope of sufficient power to make visible all the segments, they appear almost transparent, with little indication of the notches or grooves at the septa. To remedy this the following technique was devised. The worms were removed from the dishes by means of a dissecting needle and introduced into a 2 per cent. solution of formalin. As soon as bodily movements ceased the worms were transferred to a bath of India ink and then were laid out and straightened upon a glass

slide. This was accomplished very easily before the onset of rigor which would have prevented securing an accurate measure of their length. The glass slide was then placed upon the mechanical stage of a microscope equipped with a hair line in the ocular which permitted the making of an accurate count of the segments. These were clearly differentiated by the collection of carbon particles in the intersegmental notches. Two or more segment counts were made upon each worm to insure accurate results.

The worms without clitella were selected as they were brought to light from the compost heap. It should be noted that collection of newly hatched worms in the com-

TABLE 1

	Worms	No.	Range	Mean	S. D.	P. E. of S. D.	P. E. of Mean	S. D./M(100)
Segment length in mms								
	A	250	.039-.242	.1208	.038	.001	.0016	31.4
	B	250	.276-.94	.5466	.120	.0036	.005	21.9
	C	250	.49-1.278	.843	.125	.0037	.005	14.8
	D	240	.49-1.278	.849	.122	.0037	.005	14.3
Body length in mms								
	A	250	1.5- 27	12.32	4.28	.129	.183	34.7
	B	250	29- 95	57.64	12.55	.378	.535	21.7
	C	250	49-135	88.06	13.35	.403	.57	15.1
	D	240	50-135	88.60	13.15	.405	.573	14.8
Segment number								
	A	250	37-136	99.65	16.95	.511	.722	17.0
	B	250	59-125	105.5	9.55	.288	.407	9.0
	C	250	67-124	105.0	9.78	.294	.417	9.3
	D	240	67-124	104.75	9.80	.301	.426	9.3

A Newly hatched worms

B Worms without clitella

C Mature worms (all)

D Mature worms (*Helodrilus foetidus*)

post is virtually impossible. Observation of the range in length of the worms (Table 1) taken discloses that the lower extreme of this group is approximately the same as the upper extreme of the newly hatched worms. The technique of killing the worms was the same as that employed in the other group, and, as before, the worms were straightened and measured before rigor occurred. In the small worms the segments were counted by means of the microscope mentioned, while those in the larger ones were counted by using a binocular microscope. The worms with clitella were killed in a stronger solution of formalin and the segments were counted by means of the binocular microscope.

Worms with clitella were collected in the spring of 1927 between April 6 and April 27; those without clitella between May 23 and June 11.

RESULTS

In Table 1 are presented data pertaining to the length in millimeters of each segment, length of the worms in millimeters and number of segments for each selected stage of development—newly hatched, worms without clitella and mature worms. It should be noted that ten

TABLE 2
Melodrilus subrubicundus

Body length in mms	Segment number in Clitellum	Segment number ant. to Clitellum	Total segment number
79	8	21	105
78	8	21	119
67	8	21	111
72	8	21	117
49	8	21	94
83	8	21	106
81	8	21	117
79	8	21	117
79	8	21	113
92	8	21	107
Ave. 75.9	8	21	110.6

of the mature worms are of a different species (*Helodrilus subrubicundus*), the data for these being presented in Table 2 and for mature *Helodrilus foetidus* in Table 3.

TABLE 3
Helodrilus foetidus

	Range	Mean	S. D.	P. E. of S. D.	P. E. of Mean	S. D./M(100)	Correlation r P. E. of r
Segment length	.49-1.278	0.849	0.122	.0037	.0053	14.36	
Worm length	50-135	88.60	13.15	.405	.573	14.8	
Segment number	67-124	104.75	9.80	.301	.426	9.3	
Segment number in Clitellum	4- 10	7.4	0.644	.0198	.028	8.7	
Segments anterior to Clitellum	23- 28	25.629	0.661	.020	.029	2.5	
Length with segments							+ .43 ± .0355
Segments in Clit. with anterior seg.							- .40 ± .036
Segments in Clit. with total seg.							+ .078 ± .043
Segments in Clit. with length							+ .145 ± .0426

TABLE 4
P. E. OF DIFFERENCE OF THE MEANS FOR NUMBER OF SEGMENTS
OF THE WORMS

	A			B			C		
	1	2	3	1	2	3	1	2	3
A							5.35	.834	.642
B	5.85	.828	7.06						
C				.50	.583	.857			

1 Difference of means

2 Probable error of difference

3 Difference of means divided by probable error of difference

It is of interest to know that a certain definite relationship observed in the number of segments anterior to and within the clitellum first gave the clue that the selection was not homogeneous with respect to species.

Table 4 gives the probable error of the difference of the means for number of segments at different stages of development. Table 5 does the same for the means of the body lengths.

TABLE 5
P. E. OF DIFFERENCE OF MEANS FOR BODY LENGTHS

	A			B			C		
	1	2	3	1	2	3	1	2	3
A							75.74	.257	290.8
B	45.32	.788	57.5						
C				30.42	.781	38.9			

A Newly hatched worms

B Worms without clitella

C Mature worms (all)

1 Difference of means

2 Probable error of difference

3 Difference of means divided by probable error of difference

Table 6 shows the correlation between body lengths and number of segments for each selected stage of growth.

Table 7 gives Pearl and Fuller's values for *Lumbricus herculeus* Sav.

TABLE 6
CORRELATION OF BODY LENGTH IN MMS WITH
NUMBER OF SEGMENTS

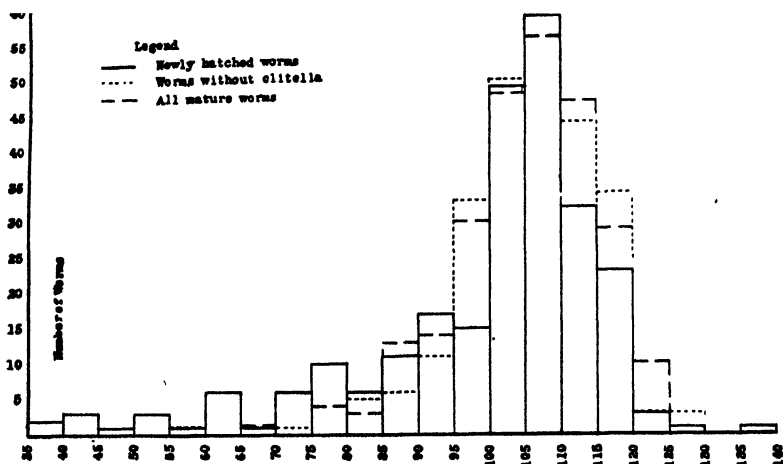
Worms	r	P. E. of r
Newly hatched worms	+ .454	± .0338
Worms without clitella.....	+ .239	± .040
Mature worms (all).....	+ .40	± .036
<i>Hclodrilus foetidus</i>	+ .43	± .0355

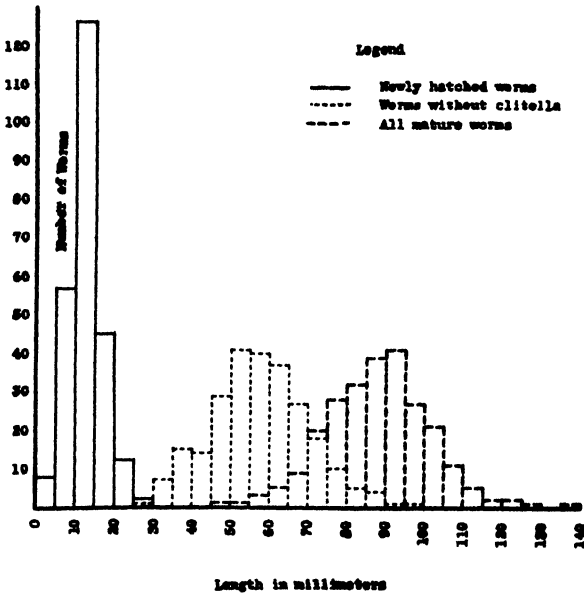
TABLE 7

Lumbricus herculeus Sav.

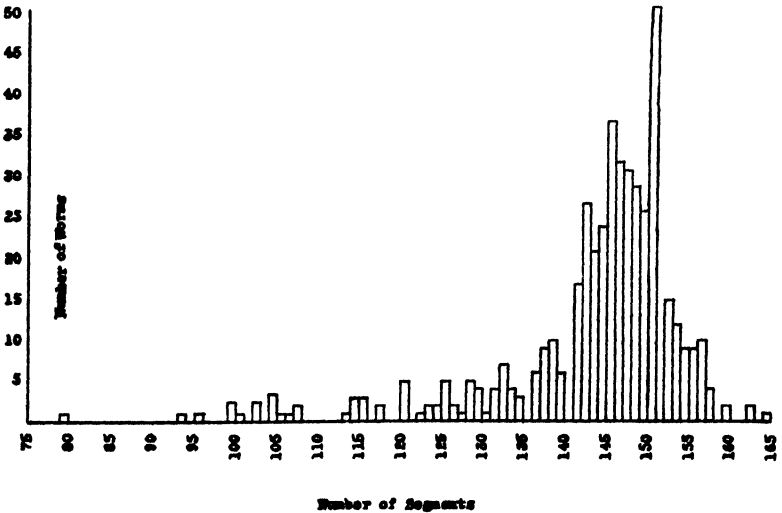
	Range	Mean	S. D.	P. E. of S. D.	P. E. of Mean	S. D./M.(100)	Correlation r P. E. of r
Segment length (mm)		1.34					
Worm length (cm)	10-30	19.17	3.077	.067	.094	16.049	
Segment number	79-164	142.71	11.850	.256	.362	8.303	
Segment number in Clitellum	6-8	6.24	0.500	.011	.015	8.019	
Segments anterior to Clitellum	29-32	30.76	0.436	.009	.013	1.416	
Length with segments							$+.260 \pm .028$
Segments in Clit. with anterior seg.							$-.629 \pm .018$
Segments in Clit. with length							$+.357 \pm .054$

Graph 1 presents the frequency distribution for number of segments at all stages of development. Graph 2 shows the frequency distribution for lengths of worms at the various stages of development. Graph 3 is a re-





production of Pearl and Fuller's showing the frequency distribution for number of segments of *Lumbricus herculeus* Sav.



DISCUSSION

In considering the relation of length to number of segments in *Lumbricus herculeus* Sav., Pearl and Fuller point out that the increase in length of an earthworm is due to the operation of two factors: (1) the growth of segments already present, and (2) the addition of new segments. To the predominance of (1) they ascribe the low correlation ($r + .260 \pm .028$) found between length and number of segments. They assume that even after sexual maturity is attained the process of segment formation continues. To this fact they ascribe the skew distribution of the number of segments and conclude that these individuals with lapse of time would add segments at such a rate that they would more nearly approach the mean.

If the data obtained in our research are examined it is found that the correlation between length and number of segments is $+ .43 \pm .0355$ (Table 6) which would seem to indicate a greater degree of relationship between these species characteristics for *Helodrilus foetidus* than is present in *Lumbricus herculeus* Sav. Does this indicate that the number of segments laid down is more important in respect to length of worm in *Helodrilus foetidus* than in the species studied by Pearl and Fuller? Or does it indicate that there is a lower variation in number of segments when maturity is attained? Inspection of Graph 1 shows that in this species we also have a skew distribution. Does this mean that those with fewest segments will add more? By what right may one assume that the skew distribution is accounted for in this fashion, namely, that in some worms the process of segmentation has ceased, that in others it is slow and in others fast? Pearl and Fuller's assumption seems to be that growth (segmentation) tends to change a skew distribution to one more nearly approaching a normal frequency distribution.

A partial answer to these questions is found when one notes the distribution graph for number of segments in

the developmental stage preceding sexual maturity. It can readily be seen that there is no appreciable difference between the range, mean and standard deviation of this selection and those for the mature worms. If one now examines the distribution for the number of segments in newly hatched worms it is apparent that there is still a skew distribution. Comparison with the distribution for mature worms reveals these differences:

(1) The range for the newly hatched worms is much greater—37–136 as compared with 67–124 (Table 1).

(2) There are not only small worms with a lesser number of segments but also ones with a greater number of segments than found in any mature worms.

Considering these facts is it not just as desirable to assume that one may account for the shortening of the range or decrease in variability of number of segments of the mature worms as being due to a process of selection eliminating those individuals deviating from an optimum range, as it is to assume an addition of new segments tending to cut off the lower end of the distribution?

It is shown that there is no significant difference between the means for worms with and without clitella, while one does exist between the newly hatched worms and those without clitella. This does not appear highly significant (7.06, Table 4), because examination of the data reveals that of the small worms 104 individuals exceed the mean of those without clitella, whereas of the latter there are 139 exceeding the mean. Similar comparison between the newly hatched worms and the mature worms reveals that 119 of the former exceed the mean for the latter, whereas 137 of the mature worms exceed the mean.

The greater dispersion of the newly hatched worms (S. D. 16.95, Table 1) may be accounted for on the basis:

(1) Of natural selection in which both extremes are eliminated;

(2) That some worms normally have a full complement of segments while others normally have relatively

fewer segments upon hatching. This would imply a higher physiological gradient in those having fewer segments so that these individuals gradually overtake the others;

(3) That the artificial environment in a very warm office room with exposure to subdued light and with material different from the normal substances surrounding the cocoons resulted in heightening the activity of developing worms to such an extent that some emerged at an earlier stage of development than would occur under normal conditions. Evidence confirmatory of this is derived from the microscopic examination of the smallest worms. They were: (a) paler in appearance; (b) less active; (c) less clearly segmented near the posterior end, thus increasing chances of error in counting; (d) susceptible to a more rapid disintegration of the cuticle by the formalin preparation.

(4) That the selection involved too small a number of individuals.

The weight of evidence seems to show that selection and not continued segmentation is the cause of lesser dispersion among mature worms than among newly hatched worms.

The means for the body lengths are significantly different (Table 1: Newly hatched worms, 12.32 mm; worms without clitella, 57.64 mm; mature worms, 88.06 mm). An inspection of Graph 2 reveals a much different situation than that found in the one portraying number of segments. The modes are not the same and the overlapping is not so great. Lack of overlapping between distributions for newly hatched worms and for worms without clitella is partially accounted for on the basis of practical difficulties in the collection of the latter. In comparing the means of the lengths for worms without and with clitella it is to be noted that a 34.5 per cent. increase in length has taken place without a significant change in number of segments.

The coefficients of variation in Table 1 show a steady decrease as the worms grow in length, seeming to indicate more rapid metabolic rates in some individuals than in others, thus leading to a closer approximation to type even though the actual range in body length increases with attainment of maturity. These coefficients of variation for lengths contrast with those for number of segments in that the latter show no steady decrease in size, only the first and second stages presenting an appreciable difference. In this case the range in segment number decreases with growth of the worms, the lower part of the distribution being affected most.

The next matter to be considered is the relation existing between the two species characteristics, body length and number of segments. If there were a perfect positive correlation we would of course find that the longer the worm the greater the number of segments. A correlation does obtain amounting to $+.40 \pm .036$ in the mature worms, $+.239 \pm .040$ in those without clitella, and $+.454 \pm .0338$ in the newly hatched worms. The most important factor in the growth of the worm is therefore the growth of the segments rather than the addition of new ones. This is also demonstrated through the 34.5 per cent. increase in the mean for the body length of the mature worms as contrasted with that of those without clitella, with no significant change in the means for the number of segments. It is worthy of note that at an intermediate stage of growth the relationship between these two species characteristics (body length and number of segments) is less than at emergence from the cocoon or at maturity. This would seem to point to a great variability in the rate of growth of the individual segments of the worms.

It should be borne in mind that in all the foregoing discussion the assumption is made that the percentages for the species remain the same throughout the heterogeneous selections. That is, since ten worms out of 250 were *Helodrilus subrubicundus* rather than *Helodrilus*

foetidus it is assumed that a like heterogeneity is found in the other selections. The assumption entails another, namely, that since the worms were collected in the same environment at the same times of the year the times of breeding and growth are similar in both species. It is said that *Helodrilus foetidus* breeds the year round. If, therefore, the other species has a limited breeding season it might well be that there would be no representatives in the non-mature groups, or representation in one but not in the other.

The second objective of the investigation was to secure data upon certain species characteristics of mature members of the species *Helodrilus foetidus* to the end that certain norms be established and the relation between various characteristics be determined. Table 3 presents these values.

It is a matter of interest to observe that there is a much greater correlation between length and number of segments in this species than in *Lumbricus herculeus* Sav. ($+.43 \pm .0355$ in the former and $+.26 \pm .028$ in the latter).

Further comparisons show:

(1) That *H. foetidus* is less than half the length of *L. herculeus* (*H. foetidus* $88.60 \pm .573$ mm; *L. herculeus* $191.71 \pm .94$ mm).

(2) That *H. foetidus* has a smaller number of segments than *L. herculeus* (*H. foetidus* $104.75 \pm .426$; *L. herculeus* $142.715 \pm .362$).

(3) That there is a greater number of segments in the clitellum of *H. foetidus* than in *L. herculeus* (*H. foetidus* $7.4 \pm .028$; *L. herculeus* $6.238 \pm .015$).

(4) That there are fewer segments anterior to the clitellum in *H. foetidus* than in *L. herculeus* (the former $25.629 \pm .029$; the latter $30.762 \pm .013$).

(5) That the correlation, although still negative, between the number of segments in clitellum and number anterior to clitellum is considerably less in *H. foetidus*

than in *L. herculeus* (the former — $.40 \pm .036$; the latter — $.629 \pm .018$).

(6) That increase in length of the worm has no very significant correlation with the number of segments in the clitellum of the species *H. foetidus* (*H. foetidus* + $.145 \pm .0426$; *L. herculeus* + $.357 \pm .054$).

(7) That in *L. herculeus* there is a greater variation with respect to length than with respect to number of segments as compared with *H. foetidus*, the coefficients of variation being: *H. foetidus*, Length 14.8, Segment number 9.3; *L. herculeus*, Length 16.049, Segment number 8.303).

From Table 2 it is apparent that *H. subrubicundus* differs considerably from *H. foetidus* and also from *L. herculeus* in the species characteristics studied. The selection is too small (ten individuals) to warrant much generalization. This species of worm seems to be smaller than *H. foetidus* with respect to body length (average = 75.9 mm). It has, however, a greater number of segments (average = 110.6). It possesses a greater number of segments in the clitellum (average = 8) than either *H. foetidus* or *L. herculeus*. And it has a lesser number of segments anterior to the clitellum than either of the other species.

This research has shown that the only satisfactory way to study changes in growth is to follow a number of worms through from the time of emergence from cocoons until they are mature, noting the changes at various stages of development. Conclusions reached, after a statistical analysis of conditions at any one stage of development, with regard to other stages, are too liable to error on account of omissions of factors which may be highly significant. The technique for measuring a worm without injuring it remains still to be devised, but until one is discovered no positive statement as to the relation of number of segments to growth in body length can be made. All that is possible at the present time is to show in which direction the evidence seems to point. It is the

hope of the writers that in further research they may obtain a definite answer to the problem.

The writers wish to acknowledge their indebtedness to Professor W. M. Barrows for valuable suggestions and to Mr. H. W. Olson for the identification of the species of worms examined in this research.

CONCLUSIONS

From the data presented it seems clear that it is not safe to assume that *H. foetidus* continues to add new segments from the time of emergence from cocoons until sexual maturity is reached. Since the data presented by Pearl and Fuller for *L. herculeus* show the same type of skew distribution with respect to segment number in that species as was found in this investigation with *H. foetidus* it seems that such an assumption is not justifiable there also.

It is just as tenable, if not more so, to assume that any difference existing between the means for number of segments of a later stage of development as compared with an earlier is due to selective factors tending to eliminate those individuals which depart from an optimal range. For

(1) There is a greater range in number of segments for newly hatched worms than for mature worms.

(2) This distribution shrinks or is cut off at both ends if that for the mature worms is compared with that for the newly hatched worms.

(3) Although there is a 34.5 per cent. increase in length from the intermediate stage (worms without clitella) to that of the mature worms, there is no significant difference between the means for the number of segments.

(4) Though there is a significant difference between the means for the number of segments in the newly hatched stage as compared with the intermediate selection (Newly hatched, Mean $99.65 \pm .722$, S. D. 16.95;

worms without clitella, Mean $105.5 \pm .407$, S. D. 9.55; P. E. of difference of means 7.06) this difference is not highly significant when one notes that while there is an increase of 5.85 segments (5.54 per cent. increase) there is an increase in body length of 78.62 per cent.

(5) This slight difference between the means may be accounted for by assuming that: (a) Selective factors have not had time to eliminate many individuals at the lower end of the distribution; (b) the laboratory technique and environment forced some worms to emerge from the cocoons before segment formation was completed.

(6) It seems significant that 104 individuals out of a selection of 250 newly hatched worms exceed the mean for number of segments of the worms without clitella, while 139 of the latter exceed the mean; 119 of the newly hatched worms exceed the mean of the mature worms, with 137 of the latter exceeding the mean.

Apparently the relation between body length and number of segments varies at different stages of development, for the correlation between the two characteristics is $+.454 \pm .0338$ for the newly hatched worms, $+.239 \pm .04$ for those without clitella, and $+.40 \pm .036$ for the mature worms. Obviously the problem can not be definitely settled until young worms with a definite number of segments can be grown to maturity and recounted.

The species *H. foetidus* as compared with *L. herculeus* manifests the following characteristics:

(1) A greater correlation between body length and segment number ($+.43 \pm .0355$).

(2) A greater number of segments in the clitellum ($7.4 \pm .028$).

(3) A smaller body length ($88.60 \pm .573$ mm).

(4) A smaller number of segments ($104.75 \pm .426$).

(5) A smaller number of segments anterior to the clitellum ($25.629 \pm .029$).

(6) A smaller negative correlation between number of segments in and anterior to the clitellum ($-.40 \pm .036$).

(7) A smaller correlation between increase in body length and segment number in the clitellum ($+.145 \pm .0426$).

(8) A smaller variation in body length in comparison to *L. herculeus* (14.8, coefficient of variation) than in segment number (9.3, coefficient of variation).

Based upon ten individuals the species *H. subrubicundus* is smaller in body length than *H. foetidus* or *L. herculeus* (average = 75.9 mm); it has a larger number of segments in the clitellum (average = 8); a lesser number of segments anterior to the clitellum (average = 21); and it possesses more segments (average = 110.6) than *H. foetidus* but less than *L. herculeus*.

THE TRIPLE-TAIL, *LOBOTES SURINAMENSIS*, ITS NAMES, OCCURRENCE ON OUR COASTS AND ITS NATURAL HISTORY

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THE NAMES OF THE TRIPLE-TAIL.

THIS deep, thick, heavily built marine percoid fish is found in all warm seas. Its marked physical make-up is responsible for the interesting names by which it is designated. Of its scientific names, the specific term *surinamensis* was given it by the old German ichthyologist, Bloch, in 1790, because his specimen had come from Surinam in northern South America. The generic term *Lobotes* was assigned by Cuvier in the second edition of his "Règne Animal."¹ It means lobed and refers to the fact that the hinder part of the body is composed of the caudal fin with the soft parts of the dorsal and anal fins projecting backward above and below, all approximately of the same length—giving the body a tri-lobed appearance. This arrangement of the three fins also gives the effect of three tails and hence is responsible also for the best known of the fish's colloquial names—"triple-tail." It is also called "steamboat" by the North Carolina fishermen in allusion to its splashing habits when caught in a seine. A seventeen-pound specimen, which I once caught at Beaufort, convinced me that it was well named. When I attempted to lift it from the seine into the boat, it spined me severely, broke away, and in its flurries to escape deluged all of us who were endeavoring to fish the bunt of the seine. The name "flasher" goes back to Theodore Gill, who in the Smithsonian Report for 1856, p. 260, wrote: "I saw a single specimen [of *Lobotes surinamensis*] in Fulton Market [New York City] last year. . . . The owner called it 'flasher,' but why so named

¹ Paris, 1829, vol. II, p. 177.

I was unable to learn." The adult fish is dull colored, even black above, and hence can not flash while dashing through the water as a white or brightly colored fish might. Perhaps Gill misunderstood the fish-dealer, who might have said "splasher," a name which as indicated would fit it admirably.

It is also called "black perch" and "sea perch" in South Carolina, and "black fish" in Louisiana. These names are fairly applicable since it is a marine percoid and generally speaking is black in color as may be seen in Fig. 1—a photograph of a living specimen about two feet long in the New York Aquarium. Off Savannah, Georgia, it is called "sunfish," probably because of its general bodily resemblance to the fresh-water fish of that name and even more because of its habit of lying on one side and basking in the sun as reported by Mr. Padgett and noted later. At Norfolk, Virginia, it is called "lump-fish" and "strawberry bass." There seems to be no particular significance in these names and I do not know why they have been applied to our fish.

GENERAL NOTES ON THE OCCURRENCE OF THE TRIPLE-TAIL ON OUR SOUTH ATLANTIC COAST

Lobotes is essentially a fish of the warm seas, and as will be shown in this section is found more abundantly as one goes south. North of Chesapeake Bay it is found only as a straggler which has been swept along in the Gulf Stream. It has been taken sparingly as far north as Massachusetts, a fine specimen having been caught off Woods Hole on September 29, 1928, and sent to the Boston Society of Natural History in whose museum it is now on display.

The earliest record for the New York region known to me dates back to Mitchill in 1815, who says that the fish in his day was uncommon in the New York market. The specimen which he described was taken in 1814 in a seine near Powles Hook on the New Jersey shore. It was 13.5 inches long and weighed one pound eleven ounces. At

the present time our fish is taken sparingly in Sandy Hook Bay. Mr. C. M. Breder, Jr., of the New York Aquarium, has listed one or two each year since 1921 as a result of his visits to the pound net fishermen. Probably a few others are taken, but the fish is only a straggler. The captures are practically confined to the warm months of July and August.

Mr. Henry W. Fowler, curator of fishes in the Academy of Natural Sciences of Philadelphia, kindly writes that he knows of but three captures of *Lobotes* on the New Jersey coast: one in 1910, and two in 1913. He has no record of its capture in Delaware Bay or on the Delaware coast.

In the Chesapeake Bay, Hildebrand and Schroeder (1928) say that the triple-tail is not uncommon. Later, when dealing with the fish from the commercial standpoint, they will be quoted in full as to its relative abundance in these waters.

At Charleston, South Carolina, Holbrook in his "Ichthyology of South Carolina"² says that the fish is found from June to September, but he gives no intimation as to its relative abundance. Farther south one would expect to find it more abundantly, and Goode (1903) relates that at Jacksonville, Florida, he examined a number of specimens. However, in four seasons' work at Tortugas, Florida, I never saw or heard of the fish. Furthermore, Professor W. H. Longley, whose acquaintance with Tortugas fishes is most extensive, writes that he has in fifteen seasons there seen but three or four specimens, and they not adults.

Later observations at points on the South Atlantic Coast, both north and south of Charleston, give fuller data as to places and relative numbers in which the triple-tail may be found. These data will now be set out.

My personal acquaintance with this fish goes back to the years 1902-11, when as investigator on fishes at the laboratory of the U. S. Bureau of Fisheries at Beaufort,

² 1855, p. 169.

North Carolina, I headed the laboratory seining crew or visited the haul-seine fishermen up Newport River almost daily during each summer. During this time I made records of the capture of a number of adult specimens. Since 1911, other adult specimens have been taken, chiefly in the pound net. Still others have been received from fishermen, and more interesting still a number of young fish have been captured. Altogether about twenty-three specimens have been listed in the laboratory records. For some of these, few data are at hand; for others the data are very complete.³ For ten of these fish we have definite records showing that they run from 18 to 28.3 inches in total length, and for six there are records of weights varying from eleven to eighteen pounds. In addition there are in the collections there three small specimens ranging from 15 to 90 mm in length. The data for these fish will, so far as possible, be tabulated later and then discussed.

From Beaufort southward the triple-tail is found in considerable abundance, and will now be discussed as a game fish. Another section will deal with it as a commercial fish on the South Atlantic Coast.

THE TRIPLE-TAIL AS A GAME FISH ON OUR SOUTH ATLANTIC COAST

My experience of occasional captures of single specimens of *Lobotes* at Beaufort had led me to believe that this fish was not only rare, but also more or less solitary in its habits. This conclusion was confirmed by the statements of other writers—the records everywhere saying “uncommon,” “few specimens ever taken,” etc. Furthermore, there was, so far as I then know, no record that this fish had ever been taken on the hook. In short, no one knew where the fish was to be found. Hence I was greatly surprised to learn from Dr. Howard A. Kelly, of Baltimore, that Dr. Charles L. Summers, of that city, who

³ For these and other valuable unpublished data in the Beaufort records, I am under very great obligations to Dr. S. F. Hildebrand, director of the Beaufort Station.

for many years had been fishing at and near Beaufort, had found a locality where triple-tails were taken in numbers on the hook. I urged Dr. Kelly to put this interesting matter on record and this he did in 1923. From his article I excerpt the following facts.

In September, 1923, Dr. Summers found these fish abundant around the hull of an old iron sidewheel steamer sunk during the Civil War about three hundred feet off the shore at a point about eight miles southwest of Beaufort Inlet. Anchoring off this wreck, Dr. Summers (who was seeking "drum," *Sciaenops ocellatus*) could see the triple-tails "disporting vigorously in the surf washing over the deck in about four feet of water at high tide." Going after them with rod and reel, in four days' fishing, nine, fifteen, eight and five (total thirty-seven) fishes, respectively, were taken, weighing from fourteen to twenty-one pounds each after they had been brought ashore more or less dried out. Much tackle was lost both because on being hooked they made for the wreck and fouled the lines and because in the open these powerful fish offered great resistance, leaping from the water like bass, never sulking but fighting from the time they were hooked until landed in the boat. This behavior is at complete variance with the statement of Jordan and Evermann (1896) that these fish are "of rather sluggish habits," as one might readily judge from their short, heavy bodies as seen in Fig. 1.

With the publication of Dr. Kelly's article, the triple-tail entered the lists of game fishes, worthy of the attention of sea anglers. However, fishing at this wreck in later years, only two or three would be taken at a time until on one trip thirteen were caught. Dr. Summers never found them other than at this particular wreck though he fished the coast thoroughly for miles above and below this spot.

So my knowledge of this fish stood until in October, 1927, when Mr. E. A. Simons, of the Charleston (South Carolina) Museum, visited the American Museum and



—Photograph by E. R. Sanborn

FIG. 1. A triple tail in the New York Aquarium (length about two feet).
Note the black color characteristic of adults.

asked me what was known about the triple-tail. In return for the little information I could give, he told me that Mr. Harry J. Padgett, master of the U. S. Engineers S. S. *Isondega*, with headquarters at Savannah, Georgia, had had extraordinary success in capturing this fish near the mouth of the Savannah River. Through Mr. Padgett's kindness I am able to set forth the following interesting data.

He catches triple-tails in considerable numbers near Savannah from June till about October 15 (the coming of cold weather), but they are most numerous in August. They are generally found about the piling of beacons or of jetties at the mouths of the rivers or smaller fresh-water streams, usually where there are eddies in which they like to stay. Ordinarily, Mr. Padgett fishes for them about four feet below the surface with a wire snell about three feet long above the hook. "The wire snell is to keep the sunfish [the name common at Savannah] from cutting the line against the barnacles on the beacon piling, since he goes for the beacon when hooked." Mr. Padgett fishes for *Lobotes* not merely for the excellent sport, but he

writes that it is delicious sea-food either baked, fried or in steaks. And Mr. Percy Viosca, Jr., director of fisheries, Louisiana Department of Conservation, informs me that in that state it "is considered one of the most excellent of food fishes and is in much demand when it can be secured."

On August 12, 1926, Mr. Padgett, while off the mouth of the Savannah River, in two hours' fishing, caught eleven triple-tails, three of which weighed 18.5 pounds each, and the whole aggregating 125 pounds. Again on August 24, 1928, Mr. Padgett and his son took a fishing party out in Calibogue Sound near Bloody Point Ranges, off the mouth of the Savannah River. In about an hour and a half the party, despite much lost tackle, caught nineteen triple-tails ranging from 5.5 to 15 pounds in weight, the whole totaling 138 pounds. These fish (and their captors) are shown in Fig. 2. For this photograph and the above valuable data, I am indebted to the interest and the courtesy of Mr. Padgett.

In answer to a recent request for further data as to the occurrence of *Lobotes* in waters under consideration, Mr.



—Photograph by courtesy of Mr. H. J. Padgett

FIG. 2. A catch of nineteen triple-tails, aggregating 138 pounds in weight, taken in Calibogue Sound, off Bloody Point Ranges, near the mouth of the Savannah River, Georgia, August 24, 1928.

Padgett and Mr. Ralph F. Rhodes, of the U. S. Engineer Office in Savannah, have kindly sent me maps of the coastal waters from the harbor of Charleston, South Carolina, to that of Fernandina, Florida. On these they have marked the localities at which they have caught triple-tails or from which they have been reliably reported. Here follow these data.

Triple-tails are found in considerable numbers around the central beacon in St. Helena Sound, formed of the confluent mouths of the Coosa and Morgan Rivers, but are not thought to go much farther in. On the other hand, but few are known to have been taken around the beacons in the mouth of Port Royal Sound. Calibogue Sound at the mouth of the Savannah River has already been referred to, but the two beacons known as Bloody Point Ranges are marked as the best fishing ground along the southeast Atlantic Coast. The fish have even been found as far up the Savannah River as the dock at Quarantine about 4.5 nautical miles inside the beacons named. It is noted that farther south the fish have been seen about the beacons in the mouth of the Altamaha River, and that they are fairly abundant around certain beacons in the center of St. Andrew Sound (the conjoined mouths of Saltilla and Cumberland Rivers). And, last of all, the beacon inside the harbor of Fernandina is marked with the statement: "It is reported that many triple-tails have been taken here."

Consideration of the facts set out above shows that the fish is fairly wide-spread from Chesapeake Bay (where it is taken in pound nets) to Fernandina, Florida. Its occurrence in considerable numbers around beacons and jetties in the sounds and mouths of rivers on our southeast coast ought to attract the attention of sea anglers, since it is evidently a fine sporting fish.

THE TRIPLE-TAIL AS A COMMERCIAL FOOD FISH ON OUR SOUTH ATLANTIC AND GULF COASTS

I have never eaten the triple-tail, and so far as I know, it was never served in "the mess" at the Beaufort Lab-

oratory, where it was my habit personally to partake of various rare fishes in order to get some idea of their palatability. However, Mr. Padgett writes that it is delicious either baked, fried or in steaks.

Its palatability and the fact that it is now known where anglers may find it in considerable numbers would lead to the conclusion that it should become of some commercial value. Hence one who has crossed the lower Chesapeake Bay (say from Cape Charles to Old Point Comfort) and has seen the large number of pound nets operated therein will not be surprised to find that our fish is caught there in sufficient numbers to make it of some commercial importance. Thus Hildebrand and Schroeder (1928) say that while not uncommon:

The lumpfish is taken in very limited numbers in the lower parts of the bay—that is, at Cape Charles and Lynnhaven Roads to Buckroe Beach, Virginia. No records of the yearly catch of this species are available. It is estimated, however, that the catch for 1922 did not exceed 1,000 pounds, all taken in pound nets [of which the lower bay is full]. A few fish are caught throughout the summer and fall, September and October yielding the largest number. . . . The size of the fish observed in the market ranged from five to twenty five pounds.

Further information as to the commercial catch of triple-tails on our coast is contained in a letter from Mr. Lewis Radcliffe, deputy commissioner of fisheries. He writes that in Virginia in 1925 the catch was only twenty-five pounds, valued at \$4.00; in North Carolina for 1927, 1,013 pounds valued at \$27. As has been shown above, the fish is found in considerable numbers in coastal waters south of Beaufort, but the Bureau of Fisheries has no reports of commercial catches in these regions. However, Mr. Radcliffe thinks these totals too small, for he adds:

In view of these records, it is quite probable that the total commercial catch is somewhat larger than the figures give and that no record has been made of the smaller catches by the fishermen as such, the fish being lumped under the heading of "miscellaneous fishes."

For the commercial catch on the Gulf Coast, the Bureau of Fisheries has no data, but Mr. Percy Viosca, Jr.,

writes that in Louisiana the triple-tail occurs out in the gulf, and that it comes in shore only in the passes at the mouth of the Mississippi River, and in the inlets between the outlying coastal islands and around these islands. Here it is occasionally taken in trawls, but mainly in trammel nets or seines. It is not a very abundant fish, the largest lots reaching New Orleans rarely exceeding 1,000 pounds. These catches are made in the summer.

THE NATURAL HISTORY OF THE TRIPLE-TAIL

Of the natural history of *Lobotes surinamensis* little is known, and that little is contained mainly in unpublished records which fortunately have come to my hand. These will be combined with all the published data and set forth herein, in the hope that thereby interest may be aroused and further information about this interesting fish gotten into the literature.

Breeding season.—Of the embryology of the triple-tail we know absolutely nothing, and even the breeding season is in doubt. Our only clue is the condition of the sexual organs. For males there is but one record. Hildebrand notes a male taken in the pound net at Beaufort on August 5, 1914, as having "testes well developed." Of female fish at Beaufort, he sends two records. The first (size and weight not noted) came from the pound net on June 20, 1916, and is recorded as having "ovaries little developed," as might be expected at that time of year. Of a very large female, 695 mm (27.4 inches) long and eighteen pounds in weight, taken in the pound net on August 25, 1914, he writes: "Roe well developed, eggs quite small, of different sizes, and very numerous. The largest are about one millimeter in diameter, and are probably not quite ripe." This is confirmed by Mr. Padgett, who writes that August-caught female fish are generally full of roe. From this it is fairly clear that the breeding season is in August or later, and that it probably takes place when the fish (presumably) go south. The sexual products are probably cast into the water, and the fertilized

eggs carried hither and yon by currents and tides—i.e., they are presumably pelagic.

Size.—Of small specimens there is fortunately no dearth, and these are very interesting. For a long time the smallest on record was one of about three inches (76 mm) said to have been collected by Professor S. F. Baird in Tuckahoe River, New Jersey. One slightly larger (3.5 inches), 90 mm, was taken by the writer in Beaufort Harbor in 1902. However, these are by no means the smallest known specimens, and I am fortunate in being able to publish the following table of small sizes ranging from 15 to 161 mm (0.57 to 6.3 inches).

A GRADED SERIES OF YOUNG TRIPLE-TAILS

No.	1	2	3	4	5	6	7	8	9	10	11	12
Mm	15	18	20	33	48	52	60	76	82	90	118	161
Inches	0.57	0.7	0.75	1.25	1.9	2.06	2.4	3.0	3.25	3.5	4.6	6.3
Locality	Cape Lookout	Port au-Prince Bay, Haiti	Panama	Port-au-Prince Bay, Haiti	Beaufort	Beaufort	Colon	Beaufort	Beaufort	Beaufort	Port-au-Prince Bay, Haiti	Port-au-Prince Bay, Haiti

No. 1, the smallest known specimen of *Lobotes surinamensis* on record, was taken at the surface of the sea at Cape Lookout, North Carolina, September 13, 1927. It was probably about a year old. Nos. 5, 6, 8, 9 and 10 were taken in Beaufort Harbor and adjacent waters. Some were collected by Dr. S. F. Hildebrand, and all were reported to me through his kindness. Nothing has ever been published on them before. There are two 76-mm fish, one collected at Beaufort in 1902 as noted above. The 60-mm specimen (No. 7) is from the Colon side of Panama. No. 3 (20 mm) is the very closely related (almost indistinguishable) *L. pacificus* from Panama Bay. Both were reported on by Meek and Hildebrand (1925). The little fish from Port-au-Prince Bay, Haiti, were collected

and described by Beebe and Tee Van (1928). Their measurements are "standard," the "over all" measurements (which apply to all the other fish) would slightly increase their lengths. Their 33-mm fish weighed only 1.2 grams. Still smaller is their 18-mm specimen—the smallest *Lobotes surinamensis* of which there is any published record up to now. However, still smaller—and the smallest ever



—Drawing by Mr W H Southwick

FIG. 3. Figure of a 15-mm (0.56 inch) triple tail taken six miles southwest of Cape Lookout, North Carolina, September 13, 1927.

recorded—is the 15-mm fish from Cape Lookout, North Carolina. It is so small that, in order to have it large enough for any detail to be recognized, it has been drawn 41 mm long and reproduced herein as Fig. 3. While small it is plainly *Lobotes*. Fig. 4 is from a photograph of No. 6, which is 52 mm in total length.

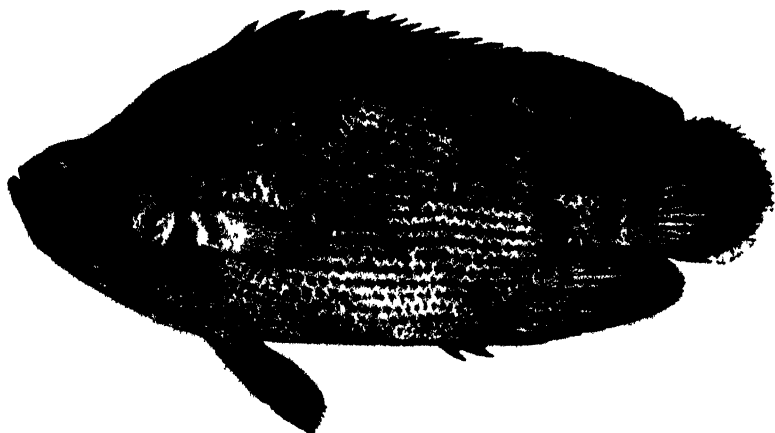


—Photograph by courtesy of Dr. S. F. Hildebrand

FIG. 4. Photograph of 52-mm triple-tail taken at Beaufort, North Carolina, July 27, 1916.

It should be emphasized that save in the matter of coloration (to be studied later) these small fish show no larval structures. All those portrayed herein, and even the little 15-mm fish, are plainly triple-tails—*i.e.*, there is no sign of metamorphosis. On this point I must differ with Jordan and Evermann (1896) who say, "Variable, the young looking quite unlike the adult."

Of forms intermediate between these small, immature fish, and the large adults presently to be referred to, quite a number have been taken but for very few are there any definite data. Hildebrand and Schroeder (1928) record a specimen from the lower Chesapeake measuring 175 mm (6.9 inches). Meek and Hildebrand (1925) figure a retouched photograph, a specimen 185 mm (7.25 inches) long from Colon. This intermediate form is reproduced herein as Fig. 5. It shows the characters of the adult fish



—After Meek and Hildebrand, 1925

FIG. 5. A *Lobotes surinamensis*, 185 mm in total length, from Colon, Panama Canal Zone.

very definitely, and is very like the smaller adults taken at Beaufort.

As to maximum size, it is everywhere stated (on the authority of J. F. Tennison Woods) that in the East Indies the triple-tail attains a length of three feet. Certain

data for our southeast Atlantic Coast fish will now be set out in tabulated form since they will give a good idea of the size attained by adult fish. Then record fish (both length and weight) will be noted.

TABLE OF SIZES AND WEIGHTS OF ADULT SOUTH ATLANTIC TRIPLE-TAILS

Number	1	2	3	4	5	6	7	8	9	10	11	12	13
Lengths in mm and inches	457 18	470 18.5	533 21	566 22.3	584 23	635 25	660 26	670 26.4	690 27.2	695 27.4	718 28.3	738 29	900 35.5
Weight in pounds							15.4	12.5		18	16	25	
Locality	Beaufort	Beaufort	Beaufort	Beaufort	New Jersey	Beaufort	Beaufort	Beaufort	Beaufort	Beaufort	Beaufort	Lower Chesapeake	Lower New York Bay

Here we have definite records of thirteen triple-tails ranging from 457 to 900 mm (18 to 35.5 inches) in length, the average being 640 mm (25.2 inches). However, it should be noted that the measurement for No. 13 is "standard"—i.e., to base of the caudal. Had the total or over all length been taken, it would have amounted to about 42 inches (1066 mm). All these fish are from Beaufort (Hildebrand's records and mine) save No. 5 from the New Jersey coast (Fowler), No. 12 from the lower Chesapeake (Hildebrand and Schroeder) and No. 13 from lower New York Bay. I have other incomplete records from Beaufort, but they need not be given since the above are thoroughly representative.

The average weight for the five Beaufort fishes listed in the table is 17.4 pounds. Dr. Summers' fish taken in the open ocean just south of Beaufort Inlet in four days' fishing were thirty-seven, varying in weight from fourteen to twenty-one pounds (lengths not given). Mr. Padgett's first report of fishing near the mouth of the Savannah River gave a total of eleven fish weighing 125 pounds—three of which weighed 18.5 pounds each. His second ac-

count is of the taking of nineteen triple-tails, ranging from 5.5 to 15 pounds and aggregating 138 pounds.

In Louisiana waters, the average weight, according to Mr. Viosca, is between seven and fifteen pounds; "large" specimens, however, weigh between twenty-five and twenty-eight pounds, and the maximum is about thirty pounds in weight and three feet in length.

Record fishes for size and weight, not only for our coast but for any and all waters so far as I know, will now be noted. No. 13 in the above table was obtained in 1925 from a pound net fisherman in lower New York Bay by Mr. C. M. Breder, Jr. This fish was kept for some time (until its death) in the New York Aquarium, where it attracted much attention. Its length (standard) of 35.5 inches is to the base of the caudal, but its total or over all length would have been about 42 inches (1066 mm). It is the largest triple-tail on record, and had its weight been taken this would have been the greatest known. The heaviest fish known to me to have been put on the scales is one of twenty-six pounds (length not recorded) taken by the late Russell J. Coles at Cape Lookout, North Carolina—where the smallest (15 mm) was also caught.

Coloration.—In my early acquaintance with the triple-tail, I found difficulty in reconciling with the descriptions in the books the fact that my 3.5-inch Beaufort specimen had white pectorals and a wide white margin on the caudal. The only explanation I could give was that this was a juvenile coloration. This was correct, as has since been found from a study of the coloration of other young and small specimens. In general it may be said that there is considerable variation in the coloration of juvenile, half-grown and adult fish, and even in fish of the same size.

For juvenile or small fish, the color may in general be described as follows: The body is brownish or greenish—darker at the back and shading off to lighter downward—with yellowish or grayish blotches. The fins are generally of the same color as the body, except the pectorals which are whitish translucent. The caudal has a variable

broad marginal band of the same color as the pectorals, especially marked in the young. The dorsal and anal fins have the general body color but are narrowly margined with lighter. Two narrow streaks on top of head between the eyes extend to nape. One streak runs from posterior margin of eye upward to nape, and another one runs from posterior margin of eye downward to the lower angle of the preopercle. These streaks are not very sharply defined. In very small specimens the brownish pigment tends to become concentrated in the centers of the scales and in certain areas, forming ill-defined cross bars and narrow longitudinal streaks along the rows of scales. There are frequently several dark spots on base of soft dorsal and anal, the latter sometimes distinctly ocellate.

The 15-mm specimen from Cape Lookout, shown in Fig. 3, is so small that only the light and dark areas can be made out. No color notes for it when fresh are on record. It is significant that not only the whole pectoral but also the *whole caudal* are white. It would be interesting to know if other specimens as small as this have white caudals. Beebe and Tee Van note that their 18 and 33 mm fish (standard length) had each a caudal with "very wide translucent terminal band."

Fig. 4 is from a photograph of the 52-mm specimen. Here the coloration is shown very accurately, since the specimen had been in alcohol too short a time (less than a week) for the color to have been materially changed. The pectoral is still transparent save at the base; the caudal, however, lacks the marginal white band. But this little fish is plainly *Lobotes*—it can not be any other fish.

Fig. 5 portrays a considerably older fish, which is beginning to take on the adult coloration. It is copied from Meek and Hildebrand's retouched photograph of a 185-mm (7.25-inch) specimen from Colon, Panama. This fish still retains the terminal white margin of the caudal, while more noticeable than in Fig. 1 of the same plate are the dark spots at the base of the soft dorsal.

That the juvenile colors above described are sometimes carried over into adult life, or may be revived on occasion, is recorded by Hildebrand of an adult male, 690 mm (27.2 inches) in total length. This fish was taken at Beaufort on July 11, 1913, and as this is (presumably) about the beginning of the breeding season for these fish in these waters, it may be conjectured that this was the nuptial coloration. (Unfortunately no examination was made to ascertain the condition of the testes.) Here are Hildebrand's carefully made but hitherto unpublished notes.

General color bronze and green above and on sides, lighter below. Closer inspection shows area around jaws and gills to be yellowish, caudal dark blue-green, dorsal and anal pinkish green and lighter than caudal. Pectorals yellowish. Pre-dorsal area a marked peacock color. Center of scales on back and sides a deep bronze-green with margin of blue. The bronze and green on back are especially marked, although so dark as to appear almost blackish.

As the fish grows older it becomes darker and rather more uniformly colored. Of the adult fish Jordan and Evermann (1896) say "Blackish above, becoming silvery gray on the sides; often blotched and tinged with yellow." Of Beaufort specimens, Smith (1907) says, "Dull black above, silvery gray on the sides and below; fins dusky; sides and fins sometimes with small yellowish blotches," while Hildebrand and Schroeder (1928) record that "Large fish examined by us in Norfolk fish markets were black everywhere on body and fins."

Such is the color of the fish (*circa* twenty-four inches long) photographed in an exhibition tank in the New York Aquarium as shown in Fig. 1. This fish's body, unfortunately, was not in exact plane with the plate in the camera. Mr. Breder tells me that the triple-tails up to about two feet long caught in Sandy Hook Bay are more or less mottled, above that size they are darker, and that they tend to become black when kept in the aquarium, as was the fish referred to. At this late day I can not clearly recall the color of my Beaufort specimens, but my recol-

lection is that at any rate they approached in color the fish in the aquarium.

From the data given it is plain that there is much difference in the coloration of the fish of any stage taken in different localities, that the juvenile coloration varies greatly from that of the adults and that the colors of the adults tend to become darker (grayish to black) as they grow older. It seems not improbable that Hildebrand's July-caught adult male was taking on the (presumed) nuptial coloration.

Food.—As to its food in nature we know little. One of 27.2 inches dissected at Beaufort contained five small menhaden (*Brevoortia tyrannus*). Beebe and Tee Van (1928) found the vertebra of a fish in their 118-mm (4.75-inch) specimen. Goode (1903) says that it feeds on small fishes and mussels, and that it readily takes the hook when this is baited with clams or with shrimp. Dr. Summers used cut mullet and squid for bait. Mr. Padgett, who has had more experience than any one else, uses shrimp, large prawns or small mullet. Specimens in the New York Aquarium are fed on shrimp, clams or cut fish. They are rather slow to feed, but when once induced to do so, they, generally speaking, eat the food fed to the other fish in the tanks with them.

Habits.—As has been noted above, my scattering catches at Beaufort (six in ten years), despite daily seinings or visits to the haul net fishermen, led me to believe that the fish was solitary. This, however, has been discounted by the experience of Dr. Summers and Mr. Padgett as set out above. It is an undoubted fact that this fish goes in schools under favorable conditions. Dr. Summers could see numbers of triple-tails about the old wreck described above. The details of the numbers taken by Dr. Summers and Mr. Padgett are set out in a previous section. Further, the latter says, "They are often seen in numbers floating at the surface on sunny days, when they are easily caught." He has sometimes seen them in schools of about a hundred in number, and he concludes

by saying that at such times "I have known them to come up close enough to the boat, floating on their sides [whence probably one of their common names, sunfish], so that one could easily strike them with an oar."

In this connection Mr. Breder tells me that their specimens in the aquarium at times seem to avoid "riding on an even keel," that they will lie for hours at an angle of 45° to the perpendicular but without any perceptible fin action. He thinks that this position is voluntarily assumed and that it is possibly done so that the fish may keep an eye on what is going on above the tank. I have elsewhere recorded similar behavior in the gray snapper (*Neomacnis griseus*) at Tortugas, Florida.

It is interesting to note that the triple-tail seems generally to be found about wrecks, the supports of beacons, the piling of jetties, sea buoys, etc. Then one asks, "Why?" Hardly for protection, since in our waters sharks are too scarce and larger teleosts too few to count much as enemies. "For food?" Possibly. Yet on this point little can be said since our knowledge of what its natural and normal food is in the wild state is almost nil. "For shade and shelter?"—probably. This is the explanation that strikes me as most plausible. In this conclusion I am influenced by the behavior of the fish noted above in the wild state and also in captivity. In the tanks of the New York Aquarium, triple-tails have proved to be timid, easily frightened, ordinarily inactive and fond of the shade. In this aquarium, above and behind each exhibition tank is a storage tank which at certain times of day casts a shadow in the rear of the lower tank. Into this shadow the triple-tail invariably retreats. To lure him out into visible range, a piece of heavy board, twenty or thirty inches long, is anchored where wanted, and under this the fish will contentedly take his place in full view of visitors. This I, myself, have seen a number of times.

That this is true of the triple-tail in the open sea is confirmed in a recent communication from Mr. Padgett in which he says, "I have seen numbers of this fish in the

vicinity of floating buoys, and also near the surface of the water in fairly large numbers around other floating objects, such as packing boxes and other débris drifting around in the ocean near the coast. When found under these conditions, the fish are easily frightened and disappear as soon as a boat approaches." Since this section was written, I have received from Mr. Percy Viosca, Jr., the following confirmatory account of the habits of the triple-tail as observed on the Louisiana and Mississippi coasts:

It is noted for its habit of lying slightly tilted on one side near the surface of the water. It is particularly fond of lurking in the shadows of floating drift, and along the outlying islands numbers of them can sometimes be seen in the shadows of fallen bushes projecting over the water where the depth is from two to three feet.

On the Mississippi coast, off Pascagoula, enterprising fishermen place the tops of pine trees out in the gulf near the islands, and fish around these with dead shrimp about eighteen inches below the surface of the water, using a long cane pole and a float. The pine tops are approached cautiously with a skiff and the bait is cast around the edge of the pine tops by means of the long pole. Sometimes as many as two or three fish are caught around each pine top.

That this habit of hiding is true of young fish as well is confirmed by Beebe and Tee Van, in the paper previously referred to. They say:

The two smaller fish were taken by scooping up Sargasso weed in a hand net, the fish being found among the fronds. . . . [They] were taken under separate patches of Sargassum, so that there can be no doubt that the young are to be found under these conditions.

While this is just what one would expect of young fish, still it ties up closely with the behavior of the adults both in captivity and in the open sea.

How these differing observations on the habits of the triple-tail are to be reconciled is not apparent. They are facts which must stand while we wait for further and more complete knowledge of the activities of this very interesting fish. It is my hope that the publication of this article may arouse interest and lead to further study of the triple-tail.

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THE INFLUENCE OF ECOLOGICAL FACTORS ON THE SIZE OF POPULATION

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THE problem of ecological distribution of organisms seems to be one of the least investigated problems of quantitative biology. It is a branch of the biology of population (*sensu* Pearl) (Pearl, 1928) and can be divided into two parts: (1) An exact study of the relationship between an abundance of given species (size of population) and ecological factors in natural conditions, and (2) an experimental study of the influence of ecological factors on the population size. We have analyzed the first of these questions in a special paper on the ecological distribution of Orthoptera (Gause, 1930). Our conclusion was that the relationship between an abundance of a species and ecological factors follows the law of Gauss. This enabled us to find the mean value for each species, which characterizes the average ecological conditions, and standard deviation, as an indicator of the ecological plasticity of the species. It was found that the ecological plasticity of a species is closely connected with the place occupied by its average conditions on the ecological scale. The more the average conditions of this or that species deviate from the middle of the scale, the less is their ecological plasticity. The object of this paper is to investigate the second group of questions, that is, the influence of ecological factors on the size of population, and to generalize the facts and data obtained in the course of the field as well as of the experimental study of the ecological distribution.

We will now examine the influence of two ecological factors, the quantity of food and the temperature, on the size of population. In both cases we are trying to express in a mathematical form the experimental data published by Chapman (1928) and Terao (1928). The first

of these authors deals with the population growth of the flour beetle (*Tribolium confusum*) in different quantities of food. He has published considerable experimental material without any attempts to analyze it mathematically. Terao studied the influence of temperature on the growth of population of the water-flea (*Moina macrocopa*). He fitted the logistic curves, representing the population growth in different conditions. The relation between the size of population and the ecological factor, however, did not interest the author.

An experiment on the influence of the quantity of food on the size of population will be the first thing we examine. We have taken the data on the population growth of *Tribolium confusum* in 16, 32, 64, and 128 grams of food (Table IV, p. 118 of Chapman's paper). These data based on considerable material are most convenient for mathematical calculations. In every case the experiment has been made on the following lines: A pair of newly emerged beetles has been placed on the food, wheat flour, which has been placed in quantities of different weight, but always in a layer two centimeters deep. The experiment has been made at a constant temperature of 27° C. and with a uniform moisture. On an average the observations have taken place every seventeenth day, when the food has been renewed and the numbers of eggs, larvae, pupae and adult beetles have been counted. This gives the size of population at different moments of its development.

The biology of the population growth of organisms has been recently investigated from different points of view by R. Pearl (1925), who succeeded in proving that the mathematical expression of the population growth is the logistic function. Recently the logistic function has been successfully applied to a whole range of biological problems (Alpatov, 1929). We fitted the curves of the growth of population in different quantities of food by using the logistic function, and obtained the data given in Table I. This table shows that the duration of the population

TABLE I

GROWTH OF POPULATION OF THE FLOUR BEETLE (*Tribolium confusum*) IN DIFFERENT QUANTITIES OF FOOD

Quantity of food in gr.	Upper asymptote	Equation of the logistic curve	The point of inflection of the curve in days
16	650	$y = \frac{650}{1 + e^{4.26903 - 0.09057x}}$	47.135
32	1025	$y = \frac{1025}{1 + e^{4.31967 - 0.08289x}}$	52.113
64	1750	$y = \frac{1750}{1 + e^{4.94020 - 0.08217x}}$	60.121
128	5000	$y = \frac{5000}{1 + e^{5.19022 - 0.05415x}}$	95.849

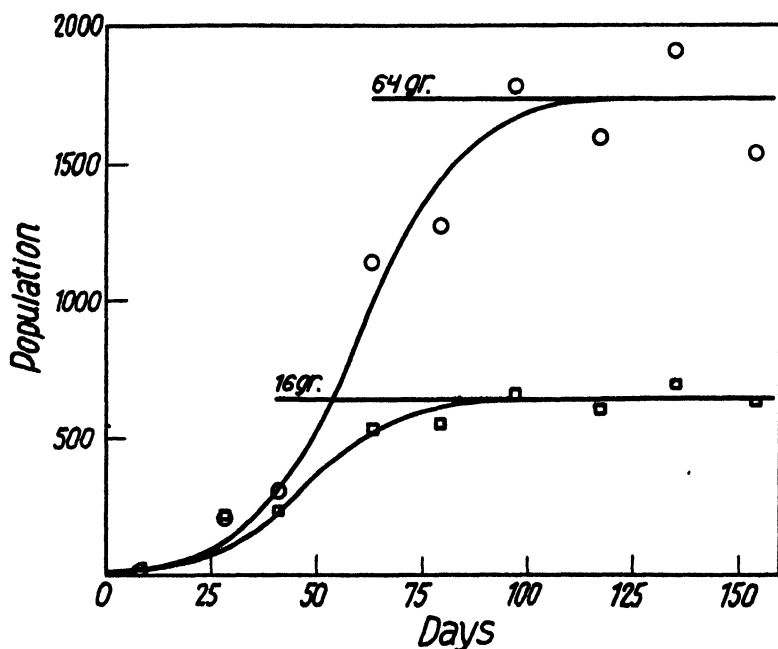


FIG. 1. Growth of population of the flour beetle *Tribolium confusum* in 16 and 64 grams of flour.

growth increases together with the increase of the quantity of food. As to the other characteristics of the curve, the upper asymptote is of especial interest for us. It shows us the asymptotic population under different conditions (Fig. 1). Evidently the asymptote is a good characteristic of the size of population in equilibrium, which corresponds to an abundance of species in nature. In field work, however, we can evaluate the abundance with but little accuracy, and study its relation not with separate ecological factors, but only with the microclimatical complexes, whereas in the simplified conditions of an experiment we are able to study with great accuracy the relationship between the size of population and the ecological factor.

The correlation between the asymptotic population and the quantity of food is represented in Fig. 2. An absence of optimal conditions and the decrease of the size of population if the conditions deviate from the optimum constitute the peculiarities of this factor. We have here the simple exponential relation between the variables, which

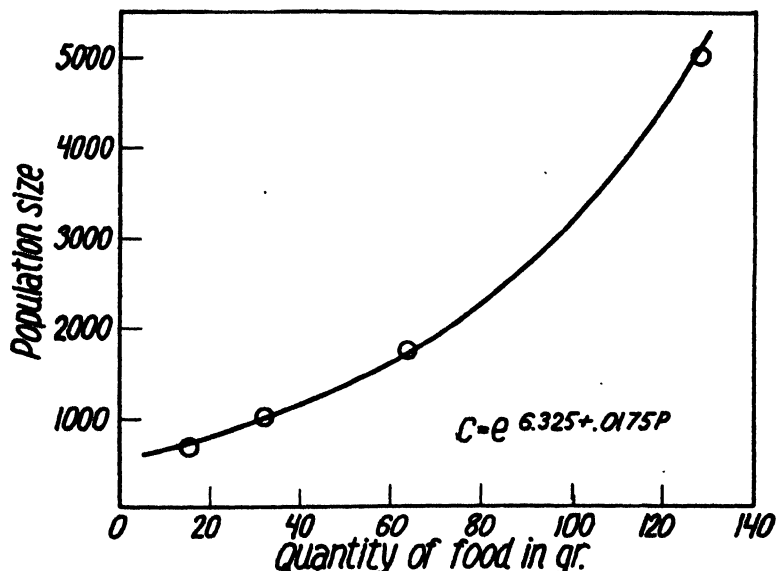


FIG. 2. The influence of quantity of food on the asymptotic population of the flour beetle *Tribolium confusum*.

can be considered as a special case of Pearson's generalized curve of probability.

As to the influence of temperature on the size of population, the experiment has been made as follows. Parthenogenetic females of the water-flea have been taken from nature and placed in vials with water containing unicellular organisms, which served as food for the water-fleas. Two cultures have been placed in the thermostat, in an average temperature of 19.8° C., two in one of 24.8° and four in one of 33.6°. Every day the water-fleas have been counted and fresh food-water has been given. Fig. 3 represents the correlation between the asymptotic pop-

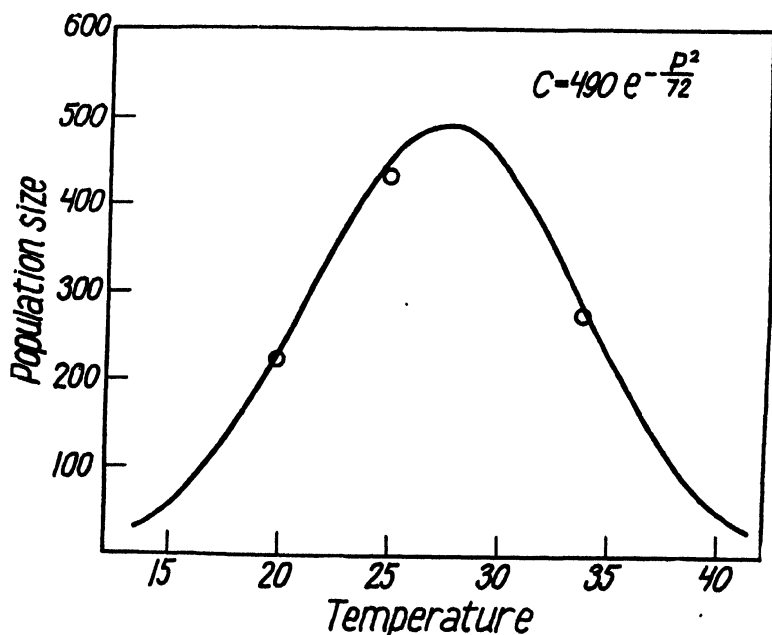


FIG. 3. The influence of temperature on the asymptotic population of the water-flea *Moina macrocopa*.

ulation and temperature. We have here the maximum population size in optimal conditions (which in this case coincide with the average ones), the size of population decreasing regularly when the conditions deviate from the optimum. The normal curve in general represents a good approximation to empirical data.

On the basis of the analyzed material we can endeavor to generalize the relationship between the size of population and ecological factors. Following Lotka (1925) we can write an equation of the kinetics of evolution, which represents the changes in time of one of the components of the system:

$$\frac{dX_1}{dt} = F_1 (X_1, X_2, \dots X_n; P_1, P_2, \dots P_j; Q_1, Q_2, \dots Q_k)$$

where $X_1, X_2, \dots X_n$ represent the masses of the given component and of others, $P_1, P_2, \dots P_j$ are parameters defining the conditions in which the growth of the component takes place and $Q_1, Q_2, \dots Q_k$ are parameters defining the character of several components. Thus the rate of growth of one of the components of the system is a function of a whole series of parameters. If we apply this equation to our example we obtain:

$$\frac{dX}{dt} = F(X, P).$$

The rate of the population growth is a function of the size of population and of the ecological factor. The simplification of the conditions in the experiment enables us to neglect other parameters.

In a case of equilibrium $F(X, P) = 0$ and $X = C$, that is, population reaches an asymptotic value. The asymptotic population depending from the ecological factor we can express in the following form:

$$C = F_1 (P).$$

The basis of the relation between the size of population and the ecological factor being the law of Gauss (generalized by Pearson), we can write for a case of normal distribution:

$$C = \frac{N}{\sqrt{2\pi}\sigma} e^{-\frac{P^2}{2\sigma^2}}$$

where σ is the standard deviation of the variation series constructed with asymptotic populations, which they reach in these or those conditions.

Taking the generalized equation of the curve of distribution we obtain the following expression for the connection between the size of population and the ecological factor:

$$\frac{1}{C} \frac{dC}{dP} = \frac{P - a}{b_0 + b_1 P + b_2 P^2}$$

where the denominator on the right side is $F_1(P)$ fractioned according to Maclaurin's series, and the constant a shows that the beginning of coordinates can be chosen at will. By using these equations we can determine the size of population with any given value of ecological factor.

The rules here established might be of great use in applied biology. The possibility of predicting *en masse* appearance of injurious animals under certain ecological conditions can facilitate the control work of pests of agriculture. The same holds true concerning wild animals useful to man.

The author is deeply indebted to Dr. W. W. Alpatov for critical suggestions and interest in the present investigation.

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ABNORMAL HERPETOLOGICAL SPECIMENS FROM SYRACUSE, NEW YORK

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IN the course of recent field work three unusual and interesting animals have been taken, the first being absolutely unique as far as can be determined from published records consulted.

I. A SPOTLESS SPOTTED SALAMANDER, *AMBYSTOMA MACULATUM* (SHAW)

Near Fayetteville, New York, a suburban village eight miles east from the center of Syracuse, lies a valley whose northern boundary is a steep hillside, stone-littered and heavily wooded with a variety of deciduous trees. An abundance of flat rock and fallen timber together with a rich humus and moist soil make this slope an excellent collecting ground for salamanders and snakes. Below, there is a level meadow with a series of cattail marshes at its upper or eastern end, and from these rises a small stream, in the course of which a good-sized, elongate pool occurs, averaging three feet in depth and ten in width. Throughout the brief extent of the brook, but especially concentrated in the pool, there is a wealth of life, among which water-snakes, green frogs, newts, spotted salamander larvae, caddis-fly larvae, water striders and pond snails stand out conspicuously. On the hillside the forms most frequently collected are milk, DeKay's and garter snakes, newts in the land stage, spotted, slimy, red-backed and dusky salamanders, and a host of worms, sow-bugs, myriapods, and cryptozoic insects, especially ground-beetles and ants.

The specimen of spotted salamander in question was discovered on May 14, 1928, beneath a flat rock about half way up the slope opposite the pool, while another and entirely ordinary individual of the same species was

found when a stone just alongside was turned. Both examples were kept alive for some time in a laboratory terrarium, but were later preserved and deposited in the U. S. National Museum, where the normal *Ambystoma* received number 76136 and the abnormal one number 76137. The typical animal was kept with the other both in the living and preserved state, because taken at the same time and place and for purposes of contrast, since it was in every way characteristic of the species.

The two captives were as alike as two peas except in the matter of coloration. They both agreed in all the well-known external structural features of *Ambystoma maculatum*, having the correct symmetry and proportions of the body and of its parts; number, length and structure of the digits; distribution of glandular pores; the skin grooves in relation with the jaws, orbit, parotid region and gular fold; a strong mid-dorsal crease, and eleven costal grooves. The detailed description of Cope (1899, pp. 56-59) applies equally well to both save for the coloration of the unique specimen. The length of the normal example was 156 mm, tail 68 mm; length of the abnormal one 162 mm, tail 80 mm. Both were adult males.

In the typical individual there were the regulation large, bright yellow spots scattered over head, body, limbs and tail, on a jet-black ground. In the other animal, the color above was everywhere a deep uniform black, lustrous when wet, and imparting a very handsome appearance. Below, the skin was somewhat lighter, being dark plumbeous gray. Along the dorsolateral margins of the tail were tiny whitish dots surrounding the pores of mucous glands, and in close view these stood out more conspicuously than in the normal form, owing to the absence of other coloration.

And yet this melano was not altogether spotless. When minutely examined, three of the customary blotches could be made out, all located at places where large, bright spots appear on the average salamander. One of these could be noted in the middle of the left shank, on the

dorsocaudal surface, and was of fairly good size, though smaller than is usual and of a faint yellow tint. The others were very small and faint indeed, requiring careful scrutiny to determine; one was situated just below the knee on the dorsal exposure of the right shank; the other appeared on the dorsolateral surface of the left parotid region. The arms, body and tail were immaculate.

When the animal was preserved in formalin these three spots became somewhat more prominent than in life, even though their color was slightly bleached, as are the large spots of a normal *A. maculatum* when thus treated. The skin took on a leaden gray hue and through it could now be made out a very fine stippling of white, giving a pepper and salt pattern, and due to the action of the preservative on the mucous secretion contained within the dermal glands.

A year of desultory correspondence and literary search has failed to bring to light any records of complete melanism in this species, and in fact has shown that abnormalities in coloration among amphibians are rare and little understood. Britcher (1899) describes the finding of a batch of about one hundred albino eggs of the spotted salamander, but these developed the typical pigmentation as they matured. Dr. Frank N. Blanchard, to whom I am indebted for several references, sent me a partially melanistic preserved example of this species, collected in White's Woods, Ann Arbor, Michigan, March 26, 1927, which I have since deposited in the U. S. National Museum, where it was given number 80337. It has somewhat less than the usual number of spots, all of which are smaller than customary, imparting a much blacker appearance than that of normal specimens, but nevertheless is spotted all over and is by no means as striking in respect to color abnormality as is the Syracuse salamander. Other references encountered had to do with experimental work in the modification of pigmentation, none of which seem to the writer to have any particular bearing on this case.

Considering that such a melanistic state in *Ambystoma maculatum* has not hitherto been reported, in spite of wide use of the species in biological investigations, the condition must be of very rare occurrence. It thus obviously can not be genetically interpreted as due to chance combination of multiple color determiners, and is too unique to permit of explanation by the smallest known percentage of crossover. Reversion seems unlikely, as jet black is not at all common among the caudata, especially the more primitive types. A new mutation, of unknown cause and behavior, appears to the writer as the most likely speculation; we know very little indeed about either melanism or color factors in salamanders.

II. A WHITE-BELLIED RED-BELLIED SNAKE, *STORERIA OCCIPITO-MACULATA* (STORER)

On July 10, 1928, the weather being clear and hot, this reptile was found beneath a piece of composition roofing which had been left lying in some deep grass along the eastern edge of the Syracuse University campus, at the foot of a hill famous locally as Mount Olympus. Numerous ground beetles, crickets, myriapods and sowbugs were noted under this and other pieces of débris, rocks and small logs in the immediate vicinity. When viewed from above during the capture, this snake appeared as a regulation individual of its species, but upon picking it up and turning it over to exhibit the red underparts to a group of students, the writer was astonished to find the belly an immaculate white. Laboratory examination showed further irregularities present in the head shields. Systematic description of the snake follows.

Body: head distinct from neck, and body quite stout; the specimen being a female distended with embryos: dorsal body scutes strongly keeled and in 15 rows: ventrals 127, the last being incomplete on the right side so that the last two resemble a divided plate: anal divided: subcaudals 42, in two rows: tail short but tapering uniformly to a fine point: L. 279 mm, T. 53 mm.

Head, normal shields: rostral and frontal single, internasals, prefrontals and supraoculars paired: nasal divided, the naris being located almost wholly in the prenasal, which is smaller than the postnasal: loreal absent: preoculars two, subequal: supralabials six, third and fourth entering eye, which is large; fifth supralabial very long and sixth next longest: infralabials seven, fifth the largest: pregenials longer and larger than postgenials, behind which comes the undivided first ventral.

Abnormal conditions were presented by the postoculars, temporals and parietals. The postoculars numbered three, subequal, on each side, instead of the regulation two; those of the left side were mutilated by some accident antedating capture. The temporals of the right side were normal, with the formula 1-2, the first being very large and long, very much pointed posteriorly and extending over nearly all of the sixth supralabial. On the left side there was a small accessory shield placed between a parietal which was hollowed-out along the middle of its lateral border, and the upper temporals. The anterior half of the lateral margin of this extra plate, directed caudoventrad, was in contact with the first temporal over the third-fourth of its extent; the posterior half, directed caudodorsad, abutted upon the upper temporal of the second row.

Coloration, living: dark pearl gray above, with a lighter vertebral band on the three central rows of scutes, bordered on each side by a darker brown stripe one scute wide. The lateral thirds of the gastrosteges, visible from above, darker gray, giving the appearance of a dark stripe along the flanks. Abdomen milky white. The three yellowish-brown nuchal spots diagnostic of this species were present and fairly well pronounced.

When preserved in formalin the upperparts took on a general tone of chestnut brown rather than gray. Examined attentively, and especially when observed through a binocular microscope, the detailed pattern was as follows: the dorsal pigment was yellowish-brown, minutely

and irregularly flecked on each scute with dark gray; about one half of the exposed surface of each scute was bordered around its periphery by a wide band of the gray color; the bulk of the fifth supralabials as well as the three nuchal blotches were yellowish-brown, on account of the suppression of the gray in these places. The flanks were so heavily speckled with the gray as to give the appearance of a nearly solid gray band, but this stippling thins out ventrally so that it disappears or is very sporadic and rare on the middle third of the gastrosteges. A narrow gray band, however, continues across most of the ventral plates in their posterior halves. Both series of labials, chin and throat speckled with the gray, more heavily forward, the chin being more gray than white, but fading out posteriorly. Head shields above chestnut brown, irregularly blotched with fuscous.

Not long after its capture this specimen shed the skin and appeared a rich and lustrous gray above, while on the abdomen the faintest tinge of opalescent rosy pink was evident. The color below would still be described as white, but it was interesting to observe this transient evidence of the normal coloration. Although in excellent condition when captured and appearing to thrive well in the terrarium all summer, six imperfectly formed young were aborted on August 6.

This species is subject to considerable variation in details of the dorsal aspect as well as in the actual color itself of the belly. Extracts from the descriptions of various observers give the dorsal ground color as olivaceous, olivaceous-gray, gray, dark gray, grayish-brown, chestnut-gray, chestnut-brown, brown, almost black. A lighter vertebral stripe is usually but not always present. This is in most cases bordered on either side by a row of fine dots that are dark brown, black, or even sometimes white, and which occasionally run together into a longitudinal stripe; but some snakes are uniform brown above with no vertebral or lateral stripes or dots. The flanks seem always to have a fairly wide band of bluish- or slate-

gray. Abdomen salmon, salmon-pink, brilliant red, bright red, bright brick red, crimson, rich crimson, vermillion. These phrases present both a general scheme of color and pattern, as well as the commoner range of variation.

Cope (1900, pp. 1004-5) lists markedly divergent individuals from Charleston and Anderson, South Carolina, in which the body is dark slate-blue except the middle third of the abdomen, which is yellowish-white. One of his specimens from Pittsburgh, Pennsylvania, is similar but with a light chestnut vertebral stripe, contrasting strongly with the ground color. Another, from Johnsonburg, Elk County, Pennsylvania, is very dark brown with the dorsal band obscure and the undersurfaces black; the throat and chin are whitish and the black is darkest in a row of spots on each side of a dozen anterior gastrosteges. Pickens (1927, p. 112) mentions "a slate-blue, yellow-bellied example from Miss Charlotte Paine, Anderson (S. C.) U. S. N. M.," presumably the same snake listed by Cope. These are the only references to a white abdomen known to the writer, nor does the literature on this snake mention such aberrant head plates as are shown by the present specimen. The case is not as unique as that of the black *Ambystoma*, but nevertheless seems worthy of recording. The value of such an individual as an exhibit is largely vitiated by the fact that the normal red color of the abdomen is bleached by preservative to a soiled grayish- or yellowish-white, hence the white-bellied example presents no unusual coloration after being a short while in formalin. This specimen was sent to the U. S. National Museum and has received number 76135.

III. AN ALBINO MUDPUPPY, *NECTURUS MACULOSUS* (RAFINESQUE)

During the first week of March, 1929, Mr. Fred R. Main, of Syracuse, was fishing through the ice of Oneida Lake near Shackelton's Point in about fifteen feet of water, and caught a perfect albino example of this salamander.

Though a large hook was embedded firmly in its throat, the animal lived for some time and created quite a bit of interest locally, being exhibited in the pharmacy window of Mr. E. H. Newell, who kindly presented the specimen to the writer. It has since been deposited in the U. S. National Museum, and numbered 80336. It may be noted as a rather amusing coincidence that all three of the abnormal animals which form the subject of this paper have specific names based on the Latin *maculatus*, spotted, and that the two amphibians fail to live up to this appellation.

The *Necturus* was an adult female measuring as follows: head, ventral aspect from lip to gular fold. 30 mm; trunk, 145 mm; tail, 65 mm; total length, 240 mm. The living creature was a very beautiful object indeed, being entirely rosy white, with bright red eyes and external gills. This rosy hue was due to capillary dermal circulation, and the only pigment appearing at any place occurred on the ends of the digits, which were tipped with chocolate brown. Not even a trace of spotting could be detected. Preserved in formalin, the animal became of the intense whiteness of the lily, including the eyes and gills.

Several zoologists and sportsmen who were consulted in person or by correspondence had vague recollections of reading or hearing about such albinos, and it seemed to the writer that he had run across an allusion to the condition here and there in print, but upon trying to trace these ideas down to definite citations, little success accrued. Apparently albinism in this species is not unknown, but only one published record came to light: Cahn and Shumway (1926, p. 107) collected two white *Necturus* larvae in Waukesha County, Wisconsin. These were preserved at the 18-mm stage, and were immaculate white on a pale yellow yolk sac. In view of the fact that the albino eggs of *Ambystoma* mentioned above gradually darkened with age, it can not be positively asserted that these white waterdog larvae would have remained colorless through-

out life, though it is very probable they would have done so. The present example is the only recorded adult known to the writer. The same authors also mention finding a number of *Necturus* larvae that were so very dark brown as easily to pass for black, and Cope (1899, p. 26) describes an entirely black individual from Ontario, Canada. The mudpuppy thus appears to be subject to more striking color variations than is usual in the caudata, but such extreme conditions in any member of the group are evidently very rare.

ADDENDA

While the foregoing article was in press another paper on albino salamander eggs has appeared (Cahn, A. R., 1930). Six clusters of these eggs were found, totaling 71, and yielding white larvae. Observations are at present continuing on the amount and rate of pigmentation developing as these larvae mature, some being raised in normal illumination and others in total darkness.

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SHORTER ARTICLES AND DISCUSSION

A MUTABLE MINIATURE GENE IN *DROSOPHILA* *MELANOGASTER*

DURING the past three years I have several times found in X-rayed material flies that showed characters of the so-called everporting type. The most common of those met with is the one that gives rise to mottled-eyed flies. Some of these have been developed into stocks and given to Mr. H. R. Bolen, who is making a special study of their genetics. Most, if not all of the mottled-eyed forms are the result of chromosome abnormalities, chiefly translocations. I have recently found a case of a mutable miniature gene that merits recording. The origin and the history to date of this case are as follows:

The original fly arose in an experiment in which virgin females were X-rayed and then crossed to yellow white lozenge miniature males. The females used belonged to a "duplication" stock, in which one X-chromosome carries the recessive factors yellow and scute, and the other X-chromosome has, in addition to yellow and scute, a small piece of the left-hand end of another X-chromosome attached to its extreme right end. This fragment carries the normal allelomorphs for yellow and scute. The sons of such females will be of two classes, namely, yellow scute and wild-type (duplication type). Mass cultures were made with the treated females, mated as stated above. These cultures yielded a total of 3,678 flies and among these were found four variant males, one of which was yellow scute miniature. Evidently, this male had received a yellow-scute X-chromosome in which a mutation to miniature had occurred.

Following our usual method of procedure in such cases, the male was mated to a yellow female with attached X-chromosomes. The culture gave 54 yellow females, 27 yellow scute miniature males and 22 yellow scute males. It was first supposed that it was a case of "fractional" mutation, that is, part of the germ cells of this male had received the mutated gene, and part had not. Such cases are sometimes found in X-rayed material. However, mass cultures were made both of the yellow scute miniature males and of the yellow scute males. The latter bred true to the wild type (normal wings), while the former gave 29

yellow scute miniature males and 23 yellow scute males. It was then apparent that the miniature was reverting back to the wild type, or normal wings, and that it was a case of a mutable gene, similar to the one found in *Drosophila virilis* by Demereč and extensively studied by him.

It was decided to keep stock of the new mutable gene, until an opportunity to make thorough tests could be found. From the tests already made, it seemed that it would be possible to maintain stock by selecting the miniature males in each generation and mating them in mass cultures to virgin yellow females with attached X-chromosomes. But in the next generation the mass culture gave 32 miniature males and only a single long winged male. If the mutable gene were not to be lost, it would be necessary to resort to individual matings. Accordingly ten paired and three mass culture matings were made. All of the paired matings and two of the three mass matings yielded miniature males only, but the third mass culture gave 26 miniature and nine normal winged males. In the next generation nine paired matings gave 427 males, all miniature winged, but there were obtained from a mass culture 231 miniatures and 55 normals.

The new miniature stock has been tested to normal miniature. All of the F_1 flies were miniature, showing that it is a true miniature. Another similar test was made by mating the yellow scute miniature males to virgin yellow white miniature females. The cultures gave 208 yellow miniature females and 205 yellow white miniature males. The long winged yellow scute males, derived by reversion from the miniature, continue to breed true to the wild-type (normal wings).

The mutable miniature gene in *Drosophila melanogaster* differs somewhat from that found in *D. virilis* by Demereč. Demereč has been able to obtain three distinct lines: (a) The alpha line, in which the gene is mutable both in the germ cells and in the somatic cells; (b) the gamma line, in which it is mutable in somatic cells only; (c) the beta line, in which miniature behaves as an almost constant gene.¹ So far I have been unable to find any evidence that mutations back to the wild-type occur in somatic cells. Demereč has also found three modifying genes,

¹ Demereč, M. 1929. The Proceedings of the National Academy of Sciences, Vol. 15, pp. 834-838.

which stimulate the mutability of the miniature gene. Our stock has not been tested for such possible modifiers.

Any case of a new mutable gene should be carefully tested and analyzed, for by so doing it may be possible to find a satisfactory explanation for this unique type of inheritance. The pressure of other work has not made this possible in the present case. I have therefore given all material to Mr. C. P. Oliver, who plans to make such a study.

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A CASE DEMONSTRATING THE ABSENCE OF SOMATIC INDUCTION IN DROSOPHILA

IN a culture obtained from the cross of an X-rayed apricot male ($w^a = \text{apricot eye}$, an allelomorph of w , white eye) by a $\overline{yy}$ female (" $\overline{yy}$ " indicates attached X-chromosomes homozygous for y , the gene for yellow body), all the males appeared phenotypically indistinguishable from white, and the females were $\overline{yy}$. There were 92 males and 103 females in all in this culture, and among them not a single exception to the above results was observed. In order to exclude the supposition of a possible contamination of the culture, or of the $\overline{yy}$ female taken for the cross with the X-rayed apricot male not having been virgin, this same male was crossed again with three other virgin $\overline{yy}$ females. All the 212 males obtained from these crosses also appeared to be phenotypically white, and the females $\overline{yy}$. Some of these males were again crossed with $\overline{yy}$ females, giving a second generation consisting of phenotypically white males and $\overline{yy}$ females. All this showed that in the apricot culture we had obtained a mutation phenotypically identical with the mutation "white."

Two unusual facts appeared in this connection. The first fact was that in a cross of this new mutation with homozygous white females, instead of our obtaining the expected pure line of white, that is, a line in which all males and females were white, we actually obtained white males, but all the females, contrary to expectation, appeared to be phenotypically like apricot. This showed that a transgene (" x ") had arisen which was not white, but some other kind of recessive. This recessive modified the gene w^a so as to produce the white

phenotype. It lay in the X-chromosome in some other position than apricot, and lost its modifying effect when in the heterozygous state. This is why females of the composition $\frac{w^a x}{w +}$, obtained from the cross of $w^a \times$ males by females homozygous for white $\left(\frac{w}{w}\right)$ produced apricot-like instead of white females $\left(\frac{w^a}{w}\right)$ females are similar in eye color to $\frac{w^a}{w^a}$.

A further investigation showed that the character "white" in our case was a complicated phenomenon, being produced by the joint action of two genes, the old gene apricot and a newly produced gene "ruby" (allelomorphic or identical with the previously known mutant of that name lying at locus 7.5 in the old standard map of the X-chromosome). The determination of this gene was made by the method of finding the per cent. of crossing-over between the gene for apricot and the gene in question. Among a total of 798 observed flies derived from females of the composition $\frac{w^a x}{cv \text{ et } f}$, 49 crossovers between w^a and x were obtained, or 6.1 per cent. From this we may conclude that the gene modifying the character apricot to white must lie at locus 7.8 ($6.1 + 1.7$, the latter being the locus of the gene apricot). This locus is very close to the previously determined locus of the known gene ruby (rb). The cross of the male carrying the new gene " x " (ruby?) with $\frac{rb}{rb}$ females, homozygous for the previously known ruby, gave a pure line of ruby. This definitely confirms the supposition of the origination, in this case, of the gene rb .

The second unusual fact is that, in the culture obtained from the cross of the male w^a by $\overline{yy}$, not only one male offspring was a mutant, as ordinarily is found to be the case at the first appearance of a mutant gene, but *all* the males of the culture at once appeared as mutants. In other words, *all* the germ cells of the X-rayed apricot male, without exception, seemed to have mutated at once, and in one direction—a case quite unthinkable from the point of view of modern genetics. Evidently the cause of this phenomenon is to be found not in simultaneous and identical mutations of all the germ cells, but in some other kind of principle. It is necessary to assume that the hereditary change in question appeared not at the time of the X-raying

- 47, 49, 51, 52, 60, 62) or less commonly, as in some species of *Palpomyia*, distinctly separated (figs. 53, 65) although they may be connected at base (fig. 66). Larvae actively swimming eel-like forms, without prolegs. Head narrow, well sclerotized; mouth parts reduced; pharyngeal skeleton with three combs; principal comb of angulus divided. Abdominal segments 3 to 7 of pupa similarly bristled; anal segment with bristles on bristle tubercle. Pupa free from larval exuviae; respiratory trumpet clavate, with numerous spiracles (**PALPOMYIA-BEZZIA GROUP**).... 29
29. Anal lobe of wing large, produced; wing broad, especially at base (figs. 36, 43); costa usually more or less produced beyond the end of the posterior branch of the radius; either one or two anterior radial cells.... 30
30. Anal lobe obtuse angled, not produced; wing usually of moderate width.... 31
30. Posterior branch of media elbowed in female; a single very narrow anterior radial cell (fig. 43); costa elongate, extending to tip of wing. Femora unarmed; fourth tarsal segment not bilobed, subcylindrical; fifth segment unarmed; claws small and equal.... **Stenoxenus**
- Posterior branch of media not elbowed in the female; one or two anterior radial cells (fig. 36); microtrichia present; medial fork broadly sessile. Anterior margin of thorax somewhat jutting over the head but not pointed. Femora unarmed, fourth tarsal segment cordate or bell-shaped, broader than long; fifth segment with strong, blunt spines (batonnets) below.... **Jenkinshelea**
31. With two anterior radial cells (fig. 39).... 32
- With but one anterior radial cell (fig. 40).... 40
32. Costa produced beyond the apex of the posterior branch of the radius (fig. 39); medial fork just sessile. Fourth tarsal segment cordate; fifth segment of fore legs swollen in both sexes as in *Clinohoelea*; claws in the female all equal and barbed.... **Neurohoelea**
- Costa not produced beyond tip of posterior branch of radius.... 33
33. Femora of fore legs armed and greatly swollen, the corresponding tibiae arched (fig. 24); hind legs of female very long, with a single long, basally barbed claw (fig. 29); claws of male small and equal; first and second pairs of the female equal and barbed. Apical third of hind femur of both sexes distinctly enlarged; fourth tarsal segments on all feet of the male and the first two pairs of the female, cordate. Antenna of male plumose. Wings of the North American species fasciate (fig. 38).... **Heteromyia**
- Fore femora not swollen, or if moderately so, fore tibia not arched.... 34
34. Claws of middle and hind legs of the female unequal and simple, or a single claw with a basal barb; fifth segment of fore tarsi inflated (fig. 27) and unarmed on all legs; fourth tarsal segment on four posterior legs deeply bilobed, each lobe terminating in a strong spine. Venation as in *Heteromyia*.... **Clinohoelea**
- Claws of hind legs of female equal, or if unequal then each with a basal barb.... 35
35. Femora and tibiae all armed with spines, some rather long, scattered irregularly; tarsi with short fourth segment which is not clearly cordate or bilobed; fifth segment unarmed; claws barbed, equal or unequal. Second radial cell much longer than the first. South America.... **Echinohoelea**
- Femora either unarmed, or if armed the spines are short and stout.... 36
36. Claws unequal in the female, at least on middle and hind feet, and all barbed; fourth tarsal segment not cordate; fifth armed below with straight, somewhat blunt, black spines (batonnets); femora with or without spines on the under side. Venation as in *Heteromyia*. Minute notal spine present as in *Palpomyia* in the North American species *D. argentalis* (Loew) and *D. annulicornis* (Malloch).... **Dicrohoelea**
- Claws of female all equal.... 37
37. Claws of female without basal barb and as long or nearly as long as the last tarsal segment; fourth segment but little longer than broad, cordate or nearly so; fifth segment of female armed with stout blunt spines (batonnets) on the under side. Oriental and South American species. The North American species assigned here by Kieffer belong elsewhere. **Homohoelea**
- Claws of female all equal and barbed, or, if not barbed, stout blunt spines (batonnets) are lacking in the female on the under side of the fifth tarsal

- segment, although stout, sharp, more or less curved bristles may be present. 38
38. Femora without strong spines below; fifth tarsal segment in the female armed with stout, blunt, black spines below (batonnets). Venation as in *Heteromyia*. Scutum without short minute, spine or tubercle anteriorly; abdomen of female without eversible glands. **Johannsenomyia**
- Femora armed with short stout black spines below. 39
39. Tarsi with fourth segment short, subcylindrical. Stout, robust species. Eyes nearly contiguous. Scutum in most cases without minute, anteriorly directed spine. Abdomen without eversible glands in the female. Fifth tarsal segment in the female with stout, black, blunt spines (batonnets) on the underside. Male antennae either with or without plume, **Sphaeromyias**
- Fourth tarsal segment cordate; fifth segment without stout, blunt spines below, but stout, sharp, somewhat curved bristles may be present. Scutum with a minute, pointed spine or tubercle anteriorly, sometimes apparently lacking. Abdomen of female with pairs of eversible glands in many species. Forceps of hypopygium with simple terminal segment; the inner caudal projecting processes either separate (fig. 53), or partly (fig. 66), or completely fused (fig. 47). Fore femora either slender or moderately swollen, with one or more short, stout spines on the lower side; middle and hind pairs slender, with or without stout spines below. Tarsal claws of moderate size, equal, with or without basal barbs. Wings rather narrow, with fine microtrichia but without macrotrichia. Venation as in *Heteromyia*, the costa extending to two-thirds the wing length or more, not produced beyond the end of the posterior branch of the radius; second anterior radial cell much longer than the first; median fork broadly sessile; anal lobe not well marked, alula absent, the short piece of wing margin between the anal lobe and the squama without fringe. **Palpomyia**
40. Wing without macrotrichia. 41
- Wing with macrotrichia (fig. 42); venation as in *Dicrobezzia* (fig. 41) or in *Probezzia* (fig. 40). Fourth tarsal segment cordate; empodium absent. In the genotype, *L. pilipennis*, from north Sweden, the fore femora are armed, the claws are simple, the fifth tarsal segment unarmed in the female, and the posterior branch of the radius ends at about two-thirds the wing length as in *Probezzia*. In the New York species, *L. unica*, the femora are unarmed, the fifth tarsal segment with stout, blunt spines below (fig. 28), the claws equal and barbed, the posterior branch of the radius elongate, disc of the scutum with short, erect bristles as in *Dicrobezzia* (fig. 23). The presence of the macrotrichia on the apical half of the wing being probably of less significance than the other characters cited the two species are not closely related. **Lasiobezzia**
41. Fourth segment of all tarsi bilobed or cordate. 42
- Fourth segment of tarsi subcylindrical; claws of female equal and with basal barb. 45
42. Mesonotum but slightly convex, with a prominent, anteriorly, and somewhat upward directed tubercle on the anterior margin (fig. 22). Abdomen elongate. Antenna of the female with short, stout, sparsely distributed hairs (fig. 20). Tarsal segments three to five very short, the fourth segment bilobed or cordate. In the genotype *C. fallax* K. from Paraguay the fifth tarsal segment of the fore legs is inflated, which is not the case in *C. flavida* Joh. from British Guiana. **Ceratobezzia**
- Mesonotum without large, stout spine on the anterior margin. 43
43. Costa of the wing and second radial cell of the female very long, reaching nearly to the wing tip (fig. 41). Dorsum of the thorax with short, sparsely distributed, erect bristles in addition to the decumbent hairs (fig. 23). Femora unarmed; fifth tarsal segment of the female with strong, blunt, black spines (batonnets) below. **Dicrobezzia**
- Costa not prolonged. Fifth tarsal segment of the female unarmed. 44
44. At least the fore femora spinose beneath; fifth tarsal segment of fore feet not inflated; claws of female equal on all legs. Venation as in *Probezzia* (fig. 40), the fork of the media narrowly sessile, the contact often punctiform. Scutum without trace of minute anterior tubercle. **Bezzia**

tinguished on principle, if only for the reason that we often have to do not only with ordinary phenotypic changes of the organism, in reaction to influences of the environment, but with definite changes in the genotype. And only in this sense can one properly speak of a unity of phenotype and genotype.

The genotype of the germ cells never manifests itself in its carrier. *It has no phenotype at all*, as long as it exists in a hidden state, as long as it does not appear. We judge the genotype of the germ cells of an observed organism only by the subsequent analysis of the phenotype of its *offspring*. The developments of the somatic and of the germ cells do not depend *genotypically* upon each other, although both come from the same root, the same fertilized egg cell, and must exist in permanent, physiological connection with one another. A genotypic change of the "root" leads to a change in both branches growing out of it; a change, however, in some part of one of these branches is not reflected correspondingly in the other.

All these facts show once more how far modern biology has gone beyond the reasonings of the Lamarekians concerning so-called "somatic induction," beyond their naïve faith in the inheritance of acquired characters.

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CHANGE IN COLOR PATTERN IN A CAPTIVE RED SQUIRREL

AN immature male red squirrel (*Sciurus hudsonicus loquax*), one of a litter of five born in captivity on August 20, 1927, showed a marked deviation from the normal coloration of this species. The mother of this litter was captured alive and uninjured at Holland, Michigan, on August 5, 1927.

The unusual coloration consisted of a number of light gray spots on the dark gray and reddish-brown body. The locations of the spots were as follows: on the neck, a small area back of each ear; a large triangular patch on the back, extending from the shoulders to a point half-way down the back of the animal; a circular spot on each side of the body, midway between the belly and the dorsal line, just anterior to the hind legs, and one on each side of the rump at the base of the tail, the latter being totally gray (Fig. 1).

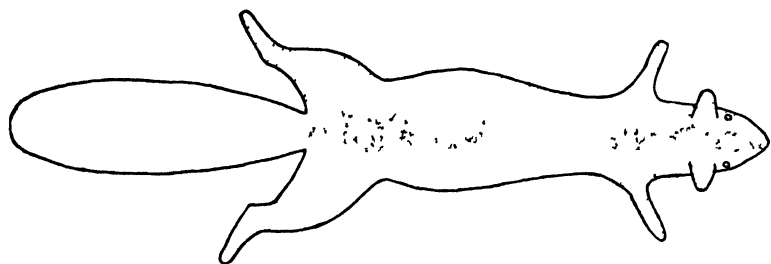


FIG. 1. Illustrating the maximum development of the light gray patches on April 4, 1928.

The grayness of the spots was found to be due to the presence of a large number of unpigmented hairs among the normally colored ones, the combination of the two giving the light gray appearance noted. This abnormality was first noticed during the spring of 1928 when the squirrel was eight months old.

At this time of the year, the other squirrels of the same litter, as well as wild squirrels in the immediate vicinity, were still in the dark red winter pelage. The odd coloration of this particular animal contrasted strongly with the normal red pelage.

As soon as discovered, this squirrel was separated from his cage-mates and observed. Drawings were made of the animal on April 4, when first discovered and separated, on April 13 and on May 6, 1928, in order to show the changes occurring in the pelage.

Previous to its isolation, this squirrel was very seldom seen, as it persisted in remaining in the nest box. When it did come out to feed, it was tormented and chased by its cage-mates until forced to seek refuge in the nest box. For this reason, the abnormal coloration was not discovered sooner. Soon after its separation, the squirrel lost most of its wildness and became more active, coming out of the nest box to feed during the daytime and to run a wheel placed in its cage.

Nine days after isolation the gray patches began to disappear. The two side patches in front of the hind legs and those at the base of the tail had entirely disappeared. The large dorsal patch had broken up into two small areas on the sides of the body and were still in the process of disappearance as they were not as light a gray as the area still remaining on the neck nor as dark a gray as the rest of the body. The two spots on the neck had fused into one narrow band extending clear across the base of the neck. The anterior half of the tail became more deeply pig-

mented and as dark a gray as the rest of the body, while the tip half still remained light gray. The disappearance of the patches was evidently accomplished by the dropping out of the unpigmented hairs and the growth of the normally colored ones.

On May 6, or thirty-two days after separation, all the light gray patches had disappeared except that on the tip of the tail, which still remained as when first discovered. When I had left for the summer, during the first part of June, this tip was still light gray. I am unable to state how long this grayness persisted, as the squirrel escaped during my absence, curtailing all further observations.

G. M. Allen,¹ in 1914, offered a theory to account for the color patterns in mammals and birds. Allen's theory may be recapitulated as follows:

Pigment production is more intense at certain definite centers on the body, and the occurrence of white or pigmentless areas is due to the restriction of pigment formation at the periphery of these centers, so that white occurs at their extremities or as breaks between contiguous color patches. There are eleven of these areas or centers on the body: one median coronal or crown patch, two aural or ear patches, two neck or nuchal patches, two scapular or shoulder patches, two side or pleural centers and two sacral or rump areas. When an animal is completely pigmented, all the centers are fully developed. When it is a total albino, none of the pigment is produced. Between these two extremes come the various "piebalds" or particolored animals that are common among domestic as well as wild animals. Allen, in his paper, gives many illustrations of this phenomenon.

This theory applies very well to my observations on the red squirrel. When the specimen was first discovered, the crown patch was well developed. The ear centers were but partially developed as they were still only a light gray. The neck areas were also not fully developed as they were also light gray. In the scapular region, the pigment production was only partially complete, for a large light gray patch separated this area from the side centers of pigmentation. Two light gray patches in the posterior portion of the body showed that the sacral center was not well developed. A patch of light gray separated the pleural from the sacral areas anteriorly, and a large posterior gray area

¹ Allen, G. M., "Pattern Development in Mammals and Birds," *AMER. NAT.*, Vol. 48, 1914, pp. 385-415, 467-484, 550-556.

suggested that the sacral pigmentation was restricted so that the entire tail region was not pigmented.

It seems that when the squirrel was given plenty of food and rest, the physiological processes improved. The squirrel tended to become more normal in action. That coloration also became more normal is evidenced by the reduction in the size and final disappearance of the light gray areas.

On May 6 and until June 2, all the light gray patches had disappeared except that on the tip of the tail, which still remained only partially pigmented. As white-tailed red squirrels are known to occur in nature (as recorded by Allen in the above-mentioned paper), it may be probable that either the sacral pigment center is the least developed of all the pigment production centers or that pigment production is first restricted in this region.

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A THUMB-TEST FOR DISTINGUISHING MAZZARD (*PRUNUS AVIUM* L.) AND MAHALEB (*PRUNUS* *MAHALEB* L.) ROOTSTOCKS

INVESTIGATIONS upon cherry orchards frequently call for the need of ascertaining whether *Mazzard* or *Mahaleb* furnish the roots of the respective trees. Well-trained nurserymen and horticulturists can quite easily distinguish the two stocks by their morphological characters. Nevertheless, a check upon these observations may be of aid in final decisions.

Small pieces of roots of approximately the same size from *Mazzard* and *Mahaleb* cherries are immersed in separate vials containing an aqueous solution of from 4 to 10 per cent. iron alum. In a few minutes the solution containing the *Mazzard* roots commences to darken. This darkening reaches its fullest intensity in from one to forty-eight hours according to the size of the pieces and the amount of liquid employed. The vial containing the *Mahaleb* roots remains practically clear and undarkened. The test has worked satisfactorily so far under all conditions of application. Roots of trees of different size and vigor have been tested at all times of the year from May

to November. The test has not been made during the winter months, as May, 1930, represents the date of its initiation.

The differential change in color is doubtlessly due to the differential quantitative presence of soluble tannic substances. From Wehmer (1), page 478, I may quote: "*P. avium* L.—Rinde n. aelteren Angaben: Gallussaeure, Tannin, neuerdings—Phlobaphen, Eisengruen Tannin—." For *P. mahaleb* no tannic substances are listed. (This is not taken to mean that *Mahaleb* is entirely devoid of tannic substances).

Czapek (2), p. 499, Vol. iii, and Abderhalden (3), page 1, Vol. 7, use "tannic substances" as a circumscriptive term. I wish it to be understood in the same way. It is therefore thought that the test can be carried out with any of the various reagents for detecting tannic substances listed in both these textbooks on biochemistry. Some of the methods have already been successfully tested by Mr. H. B. Tukey. The author wishes to express his thanks to Messrs. H. B. Tukey and K. Brase for giving the method numerous field trials.

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THE CYTOLOGICAL BASIS OF MUTATIONS¹

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It is nearly three decades since the publication of the mutation theory by de Vries, and during the intervening years the term mutation has been redefined many times in the light of increased knowledge. The processes in *Oenothera* on which de Vries based his conceptions have since been analyzed into a variety of different types of germinal change. In the meantime, the genetical work with *Drosophila* and other organisms as well as *Oenothera* has raised a great superstructure of cytogenetic fact and given us fresh insight into the architecture of the germ-plasm and its variations. It can now be recognized that a mutation is a change of *any kind* in the germinal material. Having occurred in the germ-plasm, it will be transmitted by mitotic division and inherited, provided only that the particular germ-cells in which it occurs, or their descendants, survive to take part in the production of a new individual. I shall return again later to this definition of a mutation, as some geneticists would probably not be inclined to agree with it.

During the first decade of cytological work with *Oenothera*, the counting of chromosomes made it possible to classify the mutations into four types: (1) the $(2n+1)$ forms, now called trisomics, (2) tetraploids, (3) triploids, (4) $2n$ forms in which no change in chromosome number was involved. Of the last group, a few were recognized as simple Mendelian or gene differences,

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such as *brevistylis*, *nanella* and the red factor of *rubricalyx*. The remainder, of which *rubrinervis* may be regarded as the type, were not inherited in such simple fashion, and their origin was regarded as more obscure. In this first analysis (Gates, 1915) of *Oenothera* mutations, it was naturally assumed that there would be seven ($2n + 1$) forms or primary trisomics, corresponding to the seven pairs of chromosomes. But since the study of chromosome linkage has led to the recognition that the chromosomes of a linked group are not in pairs of strict homologues, the number of possible trisomics arising from certain forms has become greater. Thus in *Oe. Lamarckiana* with a ring of 12 chromosomes and a single free pair, we may expect, as pointed out by Håkansson (1930), 13 different primary trisomic mutations, provided that all are viable; and a species such as *Oe. muricata*, *Oe. eriensis* or *Oe. novae-scotiae*, with its 14 chromosomes linked in a ring, might be expected to produce 14 more or less clearly distinguishable trisomic types. But although, as shown particularly by the work of Sheffield (1929), non-disjunction is of relatively high frequency, at least in the pollen meiosis, yet records of trisomic mutants from such wild species in cultivation are as yet remarkably few. It appears that pollen grains with 8 chromosomes are either non-functional in the great majority of cases or lose their extra chromosome in the nuclear divisions of the male gametophyte. These facts detract from the evolutionary importance of non-disjunction in the genus *Oenothera*; and since all known wild species of *Oenothera* have 14 chromosomes, we may assume that any forms which may have appeared as a result of this or other chromosome aberrations have been unable to run the gauntlet of natural selection and achieve permanency.

All modern cytogenetic studies emphasize the remarkable constancy of the germ-plasm, although this appears to be greater in some groups than in others. The chromosome linkage maps of *Drosophila*, for example, show that as regards *D. melanogaster* and *D. simulans*, the

genes occupy corresponding positions in each chromosome, except that a portion of chromosome III appears to have been reversed (see Sturtevant, 1929). Similarly in these species the evidence shows that in their whole history no genes have shifted from one chromosome to another of a different pair. In other words, there has been no translocation or crossing-over between non-homologous chromosomes since these species originated, because all the genes known occur in homologous chromosomes in the two species. It is found, however, that in *D. melanogaster* inversions of sections of chromosomes II and III are not uncommon. But they do not result in phenotypic difference or in cross-sterility.

D. melanogaster and *D. simulans* are so nearly alike that they were not recognized as distinct until 1919, and, although they will cross, the offspring are sterile. The two species show a high amount of parallel mutation, 27 of the loci that have mutated in *simulans* having also mutated in *melanogaster*. High mutability of a particular locus in one species is also correlated with high mutability of that locus in other species. Yet notwithstanding the remarkable similarity in the structure of the germ-plasm of these two species, there is a striking difference in their sex chromosomes. In *D. simulans* the Y is a straight rod about two thirds the length of the X, while in *D. melanogaster* the Y is J-shaped and longer than the X. This difference probably accounts for the interspecific sterility, and it shows that while the autosomes of these species have been undergoing a high percentage of parallel mutations their Y-chromosomes have become physically differentiated in structure through some process of which we have no present knowledge. The shortening of the Y in *simulans* and its change to a rod shape suggests that the short arm of the *melanogaster* Y has been lost, the spindle fiber attachment thus becoming terminal. This and similar evidence from *Crepis* and other organisms makes it clear that the different chromosomes of a species may undergo independent phylogenetic change, one chromosome remain-

ing stationary except for invisible gene changes while another is undergoing important structural alterations.

It is necessary to conclude that for millennia the reproductive processes of these species of *Drosophila* have been taking place with countless millions of mitotic figures in which the same spatial relationships of the germinal elements within the chromosomes have been maintained. For greater constancy or slower rates of change one must go to inorganic geological processes such as the weathering of rock and denudation of land surfaces. In the chromosomes we have some of the most complicated and labile of chemical substances, yet all the evidence shows that the structural relationships of the hereditary elements within the chromosomes are remarkably maintained.

It is now well known, however, from the work of Muller and others, that changes in the linkage maps of *Drosophila* are very frequent after exposure to X-rays. This but emphasizes the normal constancy of gene arrangement. The chromatin rearrangements so induced (Muller, 1930a) following chromosome breakage include (1) translocation, where one of the fragments becomes attached to a different chromosome, (2) inversion of a portion of a chromosome, and (3) deletion or loss of a chromosome fragment. From inversion by crossing over may arise (4) duplication of a portion of one chromosome. All these different types of displacements in *Drosophila* chromosomes have been analyzed by Muller, through the changes in crossing-over involved. It is important to note that while of common occurrence in the offspring of X-rayed animals they rarely occur in untreated material.

Although the effects of radiation are said to be most marked on rapidly dividing tissues, yet it appears probable that the compact gel state of the chromosome is less susceptible to the effects of bombardment than the loose and uncompact state of the so-called resting nucleus. The extensive investigations of Goodspeed on the germinal effects of X-rays and radium in *Nicotiana* show

that the effects on the chromosomes are similar to those in animals. Goodspeed (1930) (see Goodspeed and Avery, 1930) describes resulting fragmentation of chromosomes, with duplication or translocation by addition of fragments to other chromosomes. Such fragments may be attached by a thread to another chromosome, thus resembling in some respects a satellite. The offspring of treated plants also contain trisomic and monosomic individuals evidently resulting from non-disjunction or non-conjunction.

It is well known from the work of Hegner (1909) and others that in Chrysomelid beetles the germ-cells are set apart at a very early stage of development, during the formation of the blastoderm, and they do not begin to multiply until the gonad walls have actually been formed to receive them. Thus the Keimbahn or germ track is set apart from the soma as a separate line of cell descent almost from the beginning of development. The same is probably true, though perhaps to a less marked extent, in insects generally. The contrast with conditions in flowering plants is striking. Here we find no distinction between germ-cells and soma until we actually reach the archesporial cells which are to produce the pollen mother cells or megaspore mother cells. The Keimbahn of a flowering plant is thus vastly longer, as regards the number of cell generations intervening between the fertilized egg and the definitive germ-cells, in higher plants than in insects. Conceivably this difference in the length of the Keimbahn may be correlated with a difference in the stability of the germ-plasm in the two groups, the fossil and other evidence suggesting that species are "longer-lived" in insects than in Angiosperms.

The work on the experimental production of mutations has been especially valuable, by providing abundant mutations for analysis, in leading to a further understanding of the various kinds of mutational change which can take place. The tendency of the Morgan school, however, has been to restrict the term mutation to a single

type of change, *viz.*, gene mutations. Muller (1930*b*), for example, speaks of the "spurious mutants" of *Oenothera*. In point of fact, the first mutation discovered by de Vries was *lata*, which we now know to be a trisomic. The history of this restriction of the term has been simple. Each type of mutation which has been cytologically analyzed, so that the nature of the process which produced it could be more or less understood, has been promptly removed from the category of "true" mutations. This has been done with the trisomics, the tetraploids, triploids and now, theoretically at least, with the mutations involving changes in the linkages between chromosomes, which are believed to be due to some form of translocation. All these have been excommunicated, leaving only the gene mutations, regarding whose origin we know nothing except that they can be produced with greater frequency by the action of X-rays. To be consistent, when we know something of the nature of a gene change these also will cease to be "true" mutations. There will then be no mutations left!

Indeed, as is well known, Lotsy has advocated the view that gene mutations, since they occur in organisms whose germ-plasm is not completely homozygous, are themselves a result of hybridity. This search for germinally pure organisms is a species of Snark-hunting which a number of investigators have pursued for two decades, but it is perhaps now generally recognized that such a condition exists chiefly in the mind of the biologist and that very few truly homozygous organisms are to be found in nature. Rather must we analyze the various types of germinal change which can take place in the evolution of material which is usually germinally heterogeneous to begin with.

To me it appears therefore more consistent to recognize all these hereditary changes in the germ-plasm as mutations of different categories, due to different kinds of germinal change and having different kinds of evolutionary significance. Probably no one will now deny the fundamental importance of tetraploidy and other grades

of polyploidy in the phylogeny of flowering plants. The tetraploid *Oenothera gigas* was one of the early mutations discovered by de Vries, but when we learned something of the way in which it was produced it was relegated to the category of "chromosome aberrations." A large number of plant genera are now known in which the tetraploid and hexaploid species are just as normal and frequently just as successful in nature as the diploid. They must have arisen at some time in one of the ways in which we know that polyploids do arise. The changes involved are germinal, and it appears to me that there is no shadow of justification for excluding them from the category of mutations, especially as they have so clearly played a fundamental rôle in the evolution of many genera. Indeed, in Angiosperms according to present knowledge polyploidy is the rule and its absence the exception. These are some of the reasons why I think the term mutation should be used in the generic sense, including various classes or categories of mutational change. It is especially among students of animal genetics, where polyploidy is of minor phylogenetic importance, that the term mutation has been so unjustifiably restricted to gene mutations.

I should like now to return to chromosome linkage, which has been the subject of so much investigation and speculation in recent years. The study of intra-chromosomal or gene linkage was largely developed before linkage between chromosomes began to be studied. Gene linkage is sometimes referred to as chromosome linkage, and as it is becoming increasingly difficult to avoid ambiguity and confusion in the use of these terms I propose the word *catenation* for the physical linkage between chromosomes, causing them to remain attached in chains or rings during the essential stages of meiosis, *i.e.*, in diakinesis and usually until separation takes place in the heterotypic anaphase. The evidence, so far as it exists (Gates and Sheffield, 1929a), indicates that in somatic mitosis the chromosomes are not paired according to their linkages during meiosis. Thus in *Oc. rubricalyx*,

which has four free pairs and a ring of six in meiosis, there is no indication of four corresponding pairs in the somatic metaphase.

Catenation was first discovered in *Oenothera rubrinervis* (Gates, 1908), but the number of chromosomes in a chain was not then recognized as fixed. Cleland (1922) afterwards found in *Oe. franciscana* a ring of four chromosomes in diakinesis, although it broke up into pairs on the heterotypic spindle. Later (1924) in *Oe. franciscana sulfurea* he found a ring of 12 chromosomes and one free pair, and this time the connections between the chromosomes persisted until metaphase. Since then, a number of workers have determined the catenation or fixed linkages in many species, mutations and hybrids of *Oenothera*. It is now generally recognized that each form has its characteristic catenation, which is usually remarkably fixed and constant in successive generations, and that these linkages account for the genetic linkage which is an equally striking feature of *Oenothera*. The chromosome connections limit the freedom of distribution of chromosomes which can take place in the reduction division; but in order that this shall be effective in determining that certain chromosome combinations shall always enter the same daughter nucleus the chromosomes must also have fixed places with relation to each other in the ring or chain. Since the ring is usually arranged in zigzag form on the spindle, alternate chromosomes normally pass to the same pole and therefore belong to the same complex, adjacent chromosomes belonging to different complexes. It therefore appears that in catenated species each chromosome in the ring or chain occupies a fixed position in relation to its neighbors.

While catenation is more characteristic of *Oenothera* than of any other known genus, yet similar conditions have now been observed in other plants, and it is possible that the process has the same meaning in all. A case which appears to be of a similar kind has been recorded in the Copepod, *Diaptomus castor*, where a ring of 3 double chromosomes is figured in the heterotypic meta-

phase, the other 14 meiotic chromosome bivalents being separate (Matschek, 1910). If this case is confirmed it would dispose of Muller's (1930*b*) argument that chromosome linkage (catenation) can not be expected to take place in bisexual organisms.

A considerable number of plants are now known to form rings or chains of associated chromosomes during meiosis. Håkansson (1925) found a ring of four in the diakinesis stage of one pollen mother cell of *Godetia amoena*; Belling and Blakeslee (1926) described chains of 3-5 chromosomes in trisomic *Daturas*; Belling (1927) figured the 12 chromosomes of *Rhoeo discolor* united into a ring; Kihara (1927) described rings or chains of 4 or 6 chromosomes in *Rumex acetosella*; Stow (1927), tetrapartite and hexapartite rings and chains in *Solanum tuberosum* as well as variously composed rings in *Tradescantia*. Darlington (1929*b*) has made an analysis of ring formation in *Tradescantia* and other Commelinaceae, while Meurman (1929) described rings of 4, 6, 8 and 10 chromosomes in the tetraploid *Aucuba japonica* with 32 chromosomes. Håkansson (1929), from an examination of Hammarlund's material of *Pisum* showing unusual genetic linkage, found that in certain plants a ring of 4 chromosomes was present, although most plants had seven free pairs. Richardson (1929) has also reported a ring of 4 chromosomes in a strain of *Pisum* from Tibet, and Gairdner and Darlington (1930) a ring or chain of 4 in a certain strain of *Campanula persicifolia*.

Belling (1927), in an important paper, gave a clue to the nature of these attachments. On the basis of specific attractions between chromosomes, he concluded that similar chromosome ends remain attached to each other and that chains or rings arise through interchange of segments between non-homologous chromosomes. More recently it has been shown (Blakeslee and Belling, 1926) that the so-called B strains of *Datura stramonium* when used in crosses induce the formation of a ring of 4 chromosomes in other strains. The remarkable results obtained by identifying the extra chromosome in the

various ($2n + 1$) forms (primaries, secondaries and tertiaryes) and their crossing with B strains are of great importance in the history of genetics.

It is necessary to point out, however, that there are characteristic differences between the phenomena of catenation in *Datura* and *Oenothera*, and it is unfortunate that no detailed study of the method of synapsis in *Datura* has yet been made. *Datura* has the advantage that its chromosomes can be separated into different morphological types, while in *Oenothera* this has not hitherto been possible, although Levitsky (1929) has recently reported in *Oe. Lamarckiana* one pair of somatic chromosomes with appendages. This may correspond to the single free pair found at meiosis in this species. The other somatic chromosomes, he finds, fall into three groups: (1) three pairs with equal arms, (2) two pairs with unequal arms, (3) a smaller pair with unequal arms. Detailed study of the somatic chromosomes may thus yield a method of identifying homologous chromosomes in different *Oenothera* species.

In *Aucuba japonica* the 8 chromosomes of the haploid set are all more or less clearly recognizable by their morphological peculiarities (Meurman, 1929), so that the various chromosomes in the rings, and their position in relation to each other, can be recognized. It appears that when similar chromosomes are consecutive in the ring they usually pass to the same pole, and the very high frequency of 25 per cent. of non-disjunction is found in this tetraploid species. Lateral chiasmata, as described by Darlington, are also found, and interpreted as resulting from the inversion of a segment of a chromosome.

Of these various cases, *Tradescantia* as analyzed by Darlington (1929*b*) shows the greatest variety of conditions, including polyploidy, fragmentation of chromosomes, inversion and reduplication of chromosome segments, translocation and interchange of segments between non-homologous chromosomes, as well as the terminalization of chiasmata according to Darlington's hypothesis. This abundance of interchanges between chromosome

segments and fragments in the tetraploid *T. virginiana* is, as Darlington points out, accumulated and made possible by the fact that the species relies upon vegetative methods of reproduction. Under these conditions, the individuality of the chromosomes during meiosis is largely destroyed, and this is presumably why the species must rely upon vegetative reproduction for its continuance. It would be quite misleading to suppose that normal species with sexual reproduction could show an equal disregard of their chromosome individuality during the meiotic processes. Darlington's hypothesis thus assumes an amount of segmental interchange which is greatly in excess of that actually found in *Oenothera*, where each species, hybrid and mutant, has as a rule its fixed linkage which is rarely departed from.

Rhoeo discolor (Belling, 1927; Darlington, 1929; Kato, 1930) is the only form except *Oenothera* in which complete catenation has yet been found. These phenomena in *Oenothera* have therefore appeared in some respects unique. Before the persistence of the connection between chromosomes was recognized, the position they took up on the heterotypic spindle was attributed by me to lack of attraction between homologues. This view of course still holds, and more recently Cleland (1928) has also spoken of "disharmony" between the chromosomes, those which form pairs being regarded as relatively homozygous and those which fail to pair as heterozygous or carrying a number of different factors. I have been impressed with the fact that all the small-flowered species except *Oc. ammophila* have a complete ring of 14 linked chromosomes at diakinesis and heterotypic metaphase. It appears to be generally agreed (Gates, 1915; Boedijn, 1924; Broekens, 1925) that in the phylogeny of the genus *Oenothera* the smaller-flowered species were developed from large-flowered ancestors. The latter are found in South America and northwards into the Southern states, and we may suppose that the genus moved northwards in North America following the retreat of the ice. It was therefore suggested (Gates, 1928) that the northward

movement had been accompanied by crossing between species thus brought into touch with each other, this intercrossing leading ultimately to complete catenation of the chromosomes, since homologous chromosomes were so unlike in the hybrids that they failed to pair. Such species will breed true so long as the chromosomes occupy fixed places in the ring and so long as the homozygous recombinations are non-viable. This latter stipulation made it necessary to assume that in the hybrid species balanced lethal factors were present or that a rearrangement of certain chromosomes had taken place; otherwise they would be expected to segregate again into the parental types. Renner (1917) had assumed that in *Oe. Lamarckiana*, for example, the *gaudens* and *velans* complexes were no longer capable of producing homozygous viable types because they had been altered through exchange of factors. He also (1921) assumed that those complexes differed as regards a single pair of their chromosomes, a view which has since been given up. The view now generally adopted is that the chromosomes composing a ring are all genetically unlike, while the chromosomes which form pairs may differ in one or more genes but are nevertheless homologous in their basic structure.

While there are therefore certain difficulties with the hypothesis of catenation arising through crossing of species, yet it is not at present wholly excluded. But it probably requires to be combined with the theory involving interchange of segments between certain chromosomes in the process of meiosis in the F_1 hybrids. It can be definitely tested by determining whether crosses between species with partially catenated or wholly paired chromosomes can give rise to relatively constant forms with more or less complete catenation. No such case has yet been recorded, but crosses are now being made to test this point.

A fact frequently lost sight of in discussions of chromosome linkage (catenation) is that in all accounts of

meiosis in *Oenothera* except one or two recent ones (*e.g.*, Weier, 1930) the heavy spireme or pachynema has been recognized as a *continuous* thread without free ends, *i.e.*, as forming a closed ring from which the ring of 14 chromosomes end-to-end which is known to occur in several species is formed by constriction between the chromosomes. In some species the pachynema not only constricts at intervals to form the 14 chromosomes, but one or more free pairs of chromosomes are completely cut off by the severing of their connections with the spireme, while the remainder form a closed ring. In *Oe. rubricalyx* the four free pairs appear to be cut off simultaneously from the spireme, while in *Oe. ammophila* $\times$ (*bien-nis* $\times$ *rubricalyx*) there is some evidence (Gates and Sheffield, 1929*b*) that the three pairs may be cut off successively. If this continuous pachynema is a fact in all *Oenothera* forms, regardless of their catenation, then in this genus the chromosomes pass through a stage in which they are *all* arranged end-to-end in a closed ring. As the pachynema constricts to form the chromosomes, one or more pairs are cut off (the members of each pair being arranged tandem) while the rest of the chromosomes remain attached end-to-end because the spireme has failed to complete the constrictions between successive chromosomes. Thus the linkage of chromosomes which occurs in catenated species may be looked upon as a failure of the pachynema to complete its segmentation. It is possible that the linkages could be accounted for by a tendency for the constrictions of the pachynema to take place between similar genes. The continuity of the pachynema is thus a fact of fundamental significance in all interpretations of *Oenothera* cytology. It has been denied, we think on inadequate evidence, by Weier (1930). But the evidence on this point, from the work of Gates, Cleland, Sheffield, Håkansson, Oelkers (1926), Catchside (1930) and others is so strong that it can not be lightly set aside.

More recently the theory of catenation through translocation or exchange of segments by crossing-over be-

tween non-homologous chromosomes has been receiving increasing attention. There are many things to be said in favor of this view, but it is also not without its difficulties, although as a principle of explanation it appears capable of considerable extension. Blakeslee and Cleland (1930) have recently applied this hypothesis to the ring formation in *Oenothera*. While I have assumed (Gates, 1928) that linkage has arisen through crossing between species having certain non-homologous chromosomes, they begin with a hybrid between two forms in which certain chromosomes are unlike because an exchange of segments has taken place between two chromosomes in one of the parental forms (in accord with the hypothesis of the origin of the B forms in *Datura*). This would lead to the formation of a form having a ring of four chromosomes, and so by crosses between this form and others the catenation could be increased until forms would be produced having a ring of 14. The presence of balanced lethals in the chromosomes of the ring is necessary, since the forms must then breed true because the homozygous recombinations will be non-viable.

The essential difference between their hypothesis and my former view is that, while we both assume crossing to have occurred, my hypothesis assumed that the spireme fails to segment off the non-homologous (unpaired) chromosomes in a hybrid, while they assume that reversal of ends in a pair of chromosomes has already occurred (before crossing) through segmental interchange between non-homologues. This interchange, for which Darlington also argues on the basis of chiasmata and parasynapsis, would, if it were sufficiently frequent (as in *Tradescantia*) practically do away with chromosome individuality during the period of meiosis, as already pointed out. But in *Oenothera* the evidence is all for the fixity of any linkage once it is established, and this being the case segmental exchange between non-homologous chromosomes may be expected to be relatively rare. In

any case, crossing appears necessary for the production of the large number of phenotypically distinct species with different linkages known in *Oenothera*. In a further paper, Cleland and Blakeslee (1930) make various predictions of the catenation in hybrids between species whose chromosome linkage is already known, and the success of these predictions considerably strengthens this hypothesis.

We may briefly consider certain cases of catenation on the basis of this hypothesis, using a modified notation of Blakeslee and Cleland. Thus we may represent

<i>Oe. eriensis</i>	1.2	3.4	5.6	7.8	9.10	11.12	13.14	gamete <i>a</i>
	2.3	4.5	6.7	8.9	10.11	12.13	14.1	" <i>b</i>
<i>Oe. ammophila</i>	1.2	3.4	5.6	7.8	9.10	11.12	13.14	" <i>a'</i>
	2.3	4.5	6.7	8.9	10.11	12.1	13.14	" <i>b'</i>

where *eriensis* has a ring of 14, and *ammophila* one pair and a ring of 12. The four possible combinations of complexes would then be as follows:

(1) <i>aa'</i>	1.2	3.4	5.6	7.8	9.10	11.12	13.14	} 7 pairs
	1.2	3.4	5.6	7.8	9.10	11.12	13.14	
(2) <i>ab'</i>	1.2	3.4	5.6	7.8	9.10	11.12	13.14	} 1 pair and ring of 12
	2.3	4.5	6.7	8.9	10.11	12.1	13.14	
(3) <i>a'b</i>	1.2	3.4	5.6	7.8	9.10	11.12	13.14	} ring-14
	2.3	4.5	6.7	8.9	10.11	12.13	14.1	
(4) <i>bb'</i>	2.3	4.5	6.7	8.9	10.11	12.13—13.14		} 5 pairs and ring-4
	2.3	4.5	6.7	8.9	10.11	12.1—1.14		

Now *Oe. eriensis* and *ammophila* are closely related. Although their catenation differs, they are so much alike that many botanists would consider *eriensis* a variety or subspecies of *Oe. ammophila*, but their differences remain absolutely sharp in cultures. We must assume that the relatively homozygous recombinations *aa'* and *bb'* are non-viable in the hybrids as they are in the parents, since

these linkages do not occur in the hybrids. This is a reasonable assumption, as the species themselves are both no doubt heterozygous complex-combinations breeding true, as Hoeppener and Renner (1928) have shown to be the case in *Oe. ammophila*. Then there is the further question, why does *Oe. eriensis* $\times$ *ammophila* apparently always give one free pair and a ring of 12, like the pollen parent (seven different F_1 plants from three separate crosses were examined, Sheffield, 1929), while the reciprocal (four F_1 plants examined) always has a ring of 14, again like the pollen parent? This must be because in *eriensis* $\times$ *ammophila* only the *a* female gametes of *eriensis* function, producing the combination *ab'*, which has a ring of 12 chromosomes. Similarly in the reciprocal only the *a'* megaspore of *ammophila* combines with the *b* pollen grain complex to produce the functional combination with a ring of 14 chromosomes.

In the case of *Oe. eriensis* $\times$ *rubricalyx* the result is not so simple, since the F_1 hybrid has a different linkage from either parent. Here we have

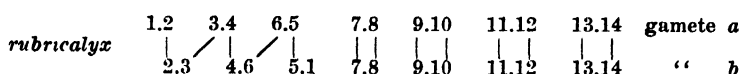
<i>Oe. eriensis</i>	1.2	3.4	5.6	7.8	9.10	11.12	13.14	gamete <i>a</i>
	2.3	4.5	6.7	8.9	10.11	12.13	14.1	" <i>b</i>
<i>Oe. rubricalyx</i>	1.2	3.4	5.6	7.8	9.10	11.12	13.14	" <i>a'</i>
	2.3	4.5	6.1	7.8	9.10	11.12	13.14	" <i>b'</i>

The four possible complex-combinations are as follows:

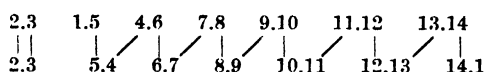
(1) <i>au'</i>	1.2	3.4	5.6	7.8	9.10	11.12	13.14	} 7 pairs
	1.2	3.4	5.6	7.8	9.10	11.12	13.14	
(2) <i>ab'</i>	1.2	3.4	5.6	7.8	9.10	11.12	13.14	} 4 pairs and ring-6
	2.3	4.5	6.1	7.8	9.10	11.12	13.14	
(3) <i>a'b</i>	1.2	3.4	5.6	7.8	9.10	11.12	13.14	} ring-14
	2.3	4.5	6.7	8.9	10.11	12.13	14.1	
(4) <i>bb'</i>	2.3	4.5	1.6	7.8	9.10	11.12	13.14	} 2 pairs and ring-10
	2.3	4.5	6.7	8.9	10.11	12.13	14.1	

Oe. rubricalyx $\times$ *eriensis* produces a crop of seedlings which are non-viable (Gates, 1930). In *eriensis* $\times$ *rubri-*

calyx none of the above four types of chromosome catenation appear, but the four F_1 plants examined all showed one pair and a ring of 12. This can only be explained on the basis of the hypothesis by assuming that two of the chromosomes in the ring of six in *rubricalyx* are differently constituted. If we write



in which chromosomes 4.5 and 6.1 are replaced by 4.6 and 5.1, then in a cross with *ericensis* the *bb'* complex-combination would give



showing the desired catenation. In this case it is not unlikely that the *bb'* combination would be viable, since the complexes of which *rubricalyx* is composed are very similar. While there is a degree of artificiality about the method, yet the scheme can be made to "work" by making certain assumptions regarding the constitution and previous translocations of the chromosomes. It is not clear, however, why an *ab'* combination, which on the above constitution of *rubricalyx* would have a ring of 14, should be non-viable. In order for the *ab'* combination to have a ring of 12, a larger amount of rearrangement between chromosome segments would be necessary.

The same situation arises in connection with *Oe. rubricalyx* $\times$ *novae-scotiae*. In this case the seed parent has a ring of 6 and the pollen parent a ring of 14, while the F_1 hybrid again shows one pair and a ring of 12.

There is much evidence, both from plant and animal hybrids, to show that the parental chromosomes maintain their morphological individuality in the hybrid soma. The theory of translocation assumes, however, that during meiosis in the hybrid the individuality is more or less broken up. In such genera as *Antirrhinum*, where inter-specific hybrids show Mendelian behavior, no such change is to be expected and no catenation is known. Catenation

will no doubt prove characteristic of certain genera, just as are the various forms of apomictic development.

We may now refer briefly to the cytological implications of the theory of translocations combined with hybridization as the cause of the catenations observed in *Oenothera*. Darlington has argued with great acuity, from the parasynapsis and chiasma behavior observed especially in various Monocotyledonous families, that the same conceptions must be of universal application, but the facts do not lend themselves easily to this interpretation. As is already well known, there is a heavy weight of evidence for telosynapsis in *Oenothera* and the evidence seems equally clear in certain other genera, such as *Lathyrus*. It would be tempting to suppose that *Oenothera* is parasynaptic, especially as parasynapsis has been described in *Godetia* (Håkansson, 1925) and *Eucharidium* (Schwemmle, 1926); but at present the cytological facts will not permit it. If Darlington's conceptions were applicable to *Oenothera*, then the connections between consecutive chromosomes in the spireme should always be double, but they have invariably been observed to be single. Their smaller size than monocotyledonous chromosomes will not suffice as an explanation of the failure to observe these double connections, for such connections have been seen and figured in the still smaller chromosomes of *Pyrus* (Darlington and Moffett, 1930).

Some years ago I expressed the view (Gates, 1922, 1924) that telosynapsis was incompatible with crossing-over, such as has been described in certain cases in *Oenothera*. Since that time, however, evidence has been adduced indicating that, for example, in *Lactuca* (Gates and Rees, 1921) and *Lathyrus* (Latter, 1926) a telosynaptic arrangement of chromosome loops could be accompanied by a possible basis for crossing-over, the chromosomes being twisted round each other in the loop stage of the spireme. It follows that telosynaptic pairing does

not exclude the possibility of crossing-over even in *Oenothera*, but it appears probable that much of such exchange in *Oenothera* is of whole chromosomes (as originally pointed out in 1908) rather than intra-chromosomal.

Meanwhile the evidence is clear that specific attractions are responsible for the formation of the chromosome rings and chains so characteristic of *Oenothera*. This evidence has recently been strengthened by the papers of Håkansson (1930a) on the catenation in a number of trisomic mutants of *Oe. Lamarckiana* in which the most frequent arrangement is an open chain of 13 and a bivalent. In another paper (1930b) he accepts the view that half-mutants such as *rubrinervis* arise through crossing-over between chromosomes widely separated in the ring, but emphasizes the difficulty pointed out by Sheffield (1929) that on this basis many other mutations should arise from *Lamarckiana*, having different linkages from the 4 pairs and ring of 6 found in *rubrinervis*. His *rubrisepala* α , with 5 pairs and a ring of 4, is, however, the only one known.

The term asyndesis has recently been used (Kuhn, 1930) for the omission of chromosome pairing, which is characteristic of the meiosis in haploids. In haploid *Oenotheras*, Davis and Kulkarni (1930) and Emerson (1929) have pointed out that the spireme stages up to diakinesis are the same as in the diploid, i.e., single threads in both. Weier (1930) has recently drawn a contrast between the conditions of the meiotic prophase in *Oe. Hookeri* with 7 chromosome pairs and *Oe. Lamarckiana* with a ring of 12. He finds parasynaptic pairing of threads in *Hookeri*, but not in *Lamarckiana* except as regards the one pair. He thus agrees that parasynapsis does not occur in *Lamarckiana* as regards the 12 chromosomes in the ring, but he calls the condition asynapsis. There appears to be no advantage in thus altering the antithesis between parasynapsis and teleosynapsis which cytologists have universally recognized for many years.

As regards Weier's observations themselves, the fixation of his material is far from satisfactory, as his own statements show. He repeatedly writes of and figures a "central coagulum" in the nucleus, which other critical investigators of the cytology of *Oenothera* have not found in their preparations. He believes that he finds in *Hookeri* a zygotene stage of pairing leptonema threads such as is not found in *Lamarckiana*, but his evidence on this point leaves much to be desired. Later, in the pachynema stage, he finds in *Hookeri* seven loops, but makes the old mistake of interpreting the two approximated arms of each loop as due to a split in the thread itself, separating the leptotene halves. This stage in which seven loops are described corresponds roughly with the brochonema stage in *Lathyrus* (Latter, 1926) although the arrangement of the loops is by no means so clear in *Oenothera*. Finally, in the second contraction stage, Weier recognizes "seven bivalent arms" projecting from the central coagulum, which he interprets as due to the "shortening and thickening of the seven bivalent threads," although the obvious interpretation appears to be that each of the "seven bivalent arms" is a pair of chromosomes end-to-end.

According to this account, *Lamarckiana* differs in having no zygonema stage except for the one pair of chromosomes. But most remarkably, in the pachynema stage he claims to find 13 arms (not loops) projecting from the "central coagulum," one of them being double and representing the free pair of chromosomes. Equally remarkable is the statement that these 13 free arms later join up by their projecting ends to form a continuous pachynema, before undergoing the second contraction and segmenting into chromosomes as described by previous investigators. In short, this paper supplies what are believed to be the theoretical cytological requirements of the moment, but there is very little evidence to substantiate his conclusions where they run contrary to previous results. We

agree with the author that in such *Oenotheras* as *Lamarckiana*, which forms rings of chromosomes, the homologues, if such can be said to exist, fail to mate. This view, however, is not new, but has been generally held for many years.

The genus *Oenothera* has served as the basis for many new conceptions in the history of genetics, and its prolificacy in that regard is probably not yet at an end. Such differences of interpretation as have existed have led always to new knowledge, and there is no doubt that the same will be true in the present instance. There is, however, a danger in making cytological observations too subservient to genetical theory, and it is for this reason that I refrain from accepting, without adequate cytological evidence, interpretations which, if true, would form an easy road through a considerable jungle of fact.

SUMMARY

The term mutation should be used in a generic sense to include inherited changes of any kind in the germ-plasm. Various types of mutations, such as trisomies, polyploids, translocations and gene mutations, can then be classified.

The remarkable constancy in the structural arrangement and spatial relationships of the elements making up the germinal material under normal conditions is emphasized.

The term *catenation* is proposed for the linkage of chromosomes in a ring or chain, now known to occur in a number of plants.

The causes of catenation, especially in *Oenothera*, are discussed, and the theory of hybridization combined with some translocations between non-homologous chromosomes is supported.

The present cytological evidence for parasynapsis in *Oenothera* is not regarded as satisfactory, although if parasynapsis occurred it would afford a basis for much at least of the chromosome ring formation in *Oenothera*.

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OENOTHERA LAMARCKIANA MUT. ACUTIFOLIA, A NEW MUTANT TYPE PRODUCED BY A GENE OUTSIDE THE FIRST LINKAGE GROUP

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IN the forty-odd years in which *Oenothera Lamarckiana* has held the attention of students of heredity it has demonstrated itself to be very singular in its behavior. Although mutations have appeared in comparatively large numbers, the vast majority of these have been "anomozygous" mutations (caused by specific irregularities in chromosome distribution) and "crossover" mutations (Shull, 1921). Mutations of these types recur year after year in experimental cultures of *Oe. Lamarckiana*. On the other hand, demonstrated gene mutations have arisen with extreme rarity during the extensive study of this species. Of these demonstrated gene mutations, only four have been shown to be inherited in typical Mendelian manner; that is, giving a 3:1 ratio by the selfing of a heterozygote and a 1:1 ratio by the backcrossing of the heterozygote to the recessive type. The other mutations have given peculiar ratios because of their linkage with the balanced lethals found in linkage-group I.

Of the four gene mutations referred to above which gave typical monohybrid ratios, one, the short-styled factor of *brevistylis* (b_r), was found by deVries in wild stock and has not appeared *de novo* in pedigreed cultures. The three remaining gene mutations behaving in typical Mendelian manner arose in the pedigreed stock of *Oe. Lamarckiana* of Dr. George H. Shull, of Princeton University. These are: Old-gold flower color of mut. *vet-aurea* (v); double flowers of mut. *supplena* (s_r), and mut. *bullata* (b_u), the latter modifying the entire aspect of the plant but most strikingly characterized by intense crinkling of the leaves (Shull, 1925, 1926, 1928a).

This paper reports the occurrence of a new gene mutation in pedigreed *Oe. Lamarckiana* and gives data to show that it is the fifth gene mutation to give typical monohybrid ratios on self-fertilization of the heterozygotes. As a name descriptive of the most obvious manifestation of the new gene, *Oe. Lamarckiana* mut. *acutifolia* (a_1), is proposed.

DESCRIPTION OF *Oe. Lamarckiana* mut. *acutifolia*,
mut. nov.

The most outstanding and consistent diagnostic character of mut. *acutifolia* is the pointed apex of the rosette leaf. Usually, at the very tip of the blade the edges are perfectly straight, coming to a fine point; in some cases the distal edges of the rosette leaf actually present a concave, recurved aspect (Fig. 1). By way of contrast the



FIG. 1. A nine weeks old rosette of *Oenothera* mut. *acutifolia*.

typical *Oe. Lamarckiana* leaf curves convexly throughout its entire length, and acutely pointed tips such as are present in the new form are rare (Fig. 2).

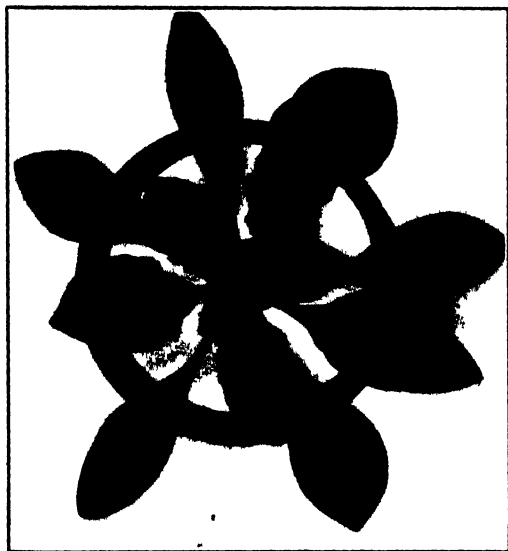


FIG. 2 A nine weeks old rosette of *Oenothera Lamarckiana*.

Another feature of the rosette leaf of *acutifolia* is its reduction in width. It is quite consistently narrower than the typical *Lamarckiana* leaf. The actual length remains the same, although relatively, because of the decrease in width, the mutant rosette leaf seems longer and more graceful.

The crinkling which appears prominently even in very young *Lamarckiana* rosettes is almost completely lacking in *acutifolia* rosettes of the same age. At a later stage, as the crinkling increases in *Lamarckiana* rosettes, the rosettes of the mutant develop a conservative amount of crinkling, although rarely equaling that manifest in rosettes of the former.

Oe. acutifolia is a weaker plant and of slower growth than *Oe. Lamarckiana*. The mutant plant, whether it be rosette or mature, is quite consistently the smaller and less vigorous. As the plants in the field approach maturity those of *acutifolia* are uniformly later by weeks in blooming. Quite an interesting feature is that the mutant plants are extremely tardy in sending up central

vertical stalks. The flowering for some time takes place exclusively on semi-procumbent side branches. Many *acutifolia* plants never develop a vertical stalk.

No apparent differences in the color of the foliage and the texture are to be found between *Oe. mut. acutifolia* and its parent species. Also the dark red spots which are so characteristic of the rosette leaf of *Oe. Lamarckiana* occur with similar frequency and degree on the rosette leaf of the mutant *acutifolia*. This new form closely parallels its parent species in floral characters and breeding phenomena. The morphological features of the flowers, the amount and fertility of the pollen, the seed output and the percentage of germination are the same in both forms.

ORIGIN OF *mut. acutifolia*

In the summer of 1928 there was placed in my hands a family (S274) of Evening Primroses, the members of which were phenotypically *Oe. Lamarckiana*. This family was a unit in a line that had been maintained for twenty-three years by Dr. Shull by appropriate crosses so that the parents of each family in the line were in no case related more closely than second cousins.

At the time, some irradiation work was being conducted in this family of *Lamarckiana*, and five plants were chosen to provide control progenies. These five plants were self-fertilized and their five progenies grown the following spring (1929). As the rosettes developed full expression of characters it was found that the progenies from three of the five plants were segregating in a 3:1 ratio for the wholly new type of rosette that forms the subject-matter of this report. The other two plants threw typical *Lamarckiana* offspring. The details are shown in Table I.

Three segregating plants out of the five tested suggested strongly that the family of *Oe. Lamarckiana* turned over to me in 1928 consisted of homozygous and

TABLE I

SELFINGS IN *Oe. Lamarckiana* FAMILY S274, THREE PLANTS OF WHICH
THREW PROGENIES WHICH SEGREGATED FOR THE NEW
MUTANT, *Oe. acutifolia*

PEDIGREE NUMBERS		Population	Non- <i>acutifolia</i>	<i>Acutifolia</i>
Plant	Family			
S274(27)	2823	89	89	0
S274(29)	2830	67	67	0
Total.....		156	156	0
S274(20)	288	80	59	21
S274(26)	2815	66	52	14
S274(33)	2837	64	54	10
Total		210	165	45
Theoretical 3: 1			157.5	52.5

heterozygous individuals, indicating that the *acutifolia* gene probably arose by a mutation which occurred in 1927, the heterozygous plant thus produced having been mated with a normal *Lamarckiana* in maintaining the cross-bred line. That this mutation did not exist in a latent condition in the several families of the cross-bred line has been shown by a series of crosses made during the summer of 1929 by Dr. Shull. Plants from five of the eight families constituting his cross-bred *Oe. Lamarckiana* strain were pollinated by mut. *acutifolia*. In each case, the F₁ progeny grown in 1930 has consisted of *Lamarckiana* and its ordinary known mutants not including mut. *acutifolia*.

BREEDING TESTS WITH *Oe. mut. acutifolia*

As shown by Table I, family 288 segregated for *acutifolia* in a fair approximation to the theoretical 3:1 ratio. This group of plants was chosen as material with which to investigate more fully the breeding behavior of *acutifolia*.

Three plants of mut. *acutifolia* were selfed to determine the pure-breeding habit of the new form. Table

II gives the results of these breedings. The three progenies totaled 71 plants; 66 were unmistakably *acutifolia*, 2 were unidentified variants such as commonly occur in *Lamarckiana* and its derivatives, and 3 were doubtful plants because of aphid attack. It may safely be said, therefore, that *acutifolia* is a pure-breeding type.

TABLE II

SELFED *Oe. mut. acutifolia*, SHOWING THE PURE-BREEDING CHARACTER OF THIS NEW MUTATION

PEDIGREE NUMBERS		Population <i>Lamarckiana Acutifolia</i>			Other variants	Unclassified (diseased)
Parent	Family					
288(17)	29181	21	0	20	0	1
288(28)	29182	12	0	11	0	1
288(33)	29183	38	0	35	2	1
Total		71	0	66	2	3

Further investigation with family 288 consisted of breeding from the phenotypic *Lamarckiana* plants, numbering 59, to ascertain if their genetic constitutions were of two kinds, namely, *A,A*, and *A,a*. To do this, 31 typical plants were selected at random and either self-fertilized or crossed with *acutifolia*. With two plants, 288(35) and 288(36), both self-fertilizations and backcrosses were made, so that as a total 23 selfings and 10 backcrosses were accomplished; in the backcrosses, *Lamarckiana* was used as female parent in three crosses, as male parent in seven crosses. No differences appeared in families from reciprocal crosses.

In a segregating family such as 288, the theoretical genotypic ratio for the *Lamarckiana* plants is 1:2. Accordingly, one third, or 10.33, of the 31 plants should be homozygous (*A,A*) and two thirds, or 20.67, should be heterozygous (*A,a*). Actually, eight plants of the 31 bred true for *Lamarckiana*, while 23 threw progenies which segregated for *Lamarckiana* and *acutifolia* either in the 3:1 ratio or the 1:1 ratio, depending on the method

of breeding. There is a departure of 2.33 plants from the theoretical, as close a fit as the small number of plants tested could be expected to give.

The data from these selfings and backcrosses are contained in Tables III, IV and V.

Table III is a tabulation of the crosses between *acutifolia* and *A₁a₁ Lamarkiana* of family 288. The number of rosettes grown was 235, of which 17 were so badly attacked by aphids as to render classification insecure. Of the 218 that could be classified 112 were non-*acutifolia* and 106 *acutifolia*. When the theoretical ratio of 109:109 is considered, the actual ratio is a remarkably close fit. On a unity basis, with the theoretical 1:1, the actual ratio is 1.05:1.

TABLE III

CROSSES OF *acutifolia* AND *A₁a₁ Lamarkiana* PLANTS IN SEGREGATING FAMILY 288

PARENTS		Family	Population	Non- <i>Acutifolia</i>		Unclassified (diseased)
Female <i>Acutifolia</i>	Male <i>Lamarckiana</i>			<i>Acutifolia</i>	<i>Acutifolia</i>	
288(15)	288(12)	291	13	4	9	0
288(15)	288(13)	292	2	1	1	0
288(15)	288(60)	293	9	3	6	0
288(15)	288(60)	29186	19	8	10	1
288(26)	288(75)	295	3	2	1	0
288(28)	288(36)	296	5	2	3	0
288(28)	288(36)	29188	5	3	2	0
288(28)	288(37)	297	8	2	6	0
288(28)	288(37)	29189	29	14	12	3
<i>Lamarckiana Acutifolia</i>						
288(43)	288(22)	298	13	6	7	0
288(43)	288(22)	29190	35	16	12	7
288(47)	288(15)	299	6	1	5	0
288(47)	288(15)	29191	5	4	1	0
288(48)	288(22)	2910	16	12	4	0
288(48)	288(33)	2911	11	6	5	0
288(48)	288(33)	29192	56	28	22	6
Total	235			112	106	17
Expected	1:1			109	109	

The progenies from sixteen self-fertilized heterozygotes are recorded in Table IV. Of 887 rosettes comprising the total population, many plants suffered from the attacks of aphids and 37 had to remain unclassified. The remaining 850 plants split into 668 non-*acutifolia* : 182 *acutifolia*. On a 3:1 basis, the numbers should have been 637.5 and 212.5, respectively. The actual ratio reduces to 3.67:1.

TABLE IV
SELFED A_1a_1 *Lamarckiana* IN SEGREGATING FAMILY 288

PEDIGREE NUMBERS		Population Non- <i>acutifolia</i>		<i>Acutifolia</i>	Unclassified (diseased)
Parent	Family				
288(36)	29194	45	37	8	0
288(37)	29195	39	31	6	2
288(39)	29196	50	32	8	10
288(40)	29197	62	45	14	3
288(41)	29198	17	12	5	0
288(46)	29199	56	47	9	0
288(51)	29200	33	27	6	0
288(52)	29201	79	56	20	3
288(56)	29203	116	93	21	2
288(58)	29205	69	54	14	1
288(61)	29207	70	54	16	0
288(63)	29209	43	33	10	0
288(69)	29213	64	42	10	12
288(73)	29214	24	17	7	0
288(74)	29215	59	41	18	0
288(77)	29216	61	47	10	4
Total 887			668	182	37
Expected 3:1			637.5	212.5	

Table V contains the individuals in the segregating family 288, which by breeding results were proved to have the genetic constitution A_1A_1 . The progenies of nine plants are represented in the table. The total population is 530 plants, 21 of which are mutants and variations common to *Lamarckiana* pedigrees. The remainder (509 plants) consist of 507 rosettes of *Oe. Lamarckiana* and two rosettes of *acutifolia*. These last-

named undoubtedly represent contamination at some stage in the cultural operations attending the growing of the plants. They need not affect the validity of the families in which they appear.

TABLE V

A₁A₁ *Lamarckiana* PLANTS IN SEGREGATING FAMILY 288. (ALL FAMILIES EXCEPT 294 AND 29187 ARE DERIVED FROM SELFINGS.)

PARENTS		Family	Population	<i>Lamarckiana</i>	<i>Acutifolia</i>	Other variants
Female	Male					
288(17)	288(35)	294	23	22	0	1
288(17)	288(35)	29187	40	39	0	1
288(35)	self	29193	32	31	0	1
288(53)	"	29202	64	63	0	1
288(57)	"	29204	104	100	0	4
288(59)	"	2930	12	8	0	4
288(59)	"	29206	9	8	0	1
288(62)	"	29208	80	78	1*	1
288(65)	"	2935	8	7	0	1
288(65)	"	29210	32	31	0	1
288(66)	"	29211	47	44	0	3
288(68)	"	29212	79	76	2*	2
Total			530	507	2*	21

* Undoubtedly contamination.

All families in Table V except 294 and 29187 were derived from self-fertilizations. These particular families, 294 and 29187, may be cited as showing the complete recessiveness of mut. *acutifolia*, as the parents of each were *Lamarckiana* (proved to be (*A₁A₁*)) and *acutifolia* (*a₁a₁*). The F₁'s (*A₁a₁*) are as follows: Family 294 consists of 23 plants, all *Lamarckiana* as the variant listed in the table is merely a normal *Lamarckiana* rosette with two growing points; family 29187 consists of 40 plants, 39 of which are *Lamarckiana*, the variant listed being *Oe. mut. pulla* (one of the recurring trisomics).

DISCUSSION

The breeding results presented in the foregoing tables establish the following points concerning the heredity of

Oe. mut. acutifolia: (1) that the characters of this new form are the expression of a single gene, a_r , which arose by a mutation presumably in 1927; (2) that *acutifolia* is a pure-breeding type; (3) that it is completely recessive when mated with its parent species, *Oe. Lamarckiana*; (4) that a typical 3:1 ratio is obtained in the progeny of a selfed heterozygote ($A_r a_r$) of *Lamarckiana* phenotype, and (5) that the dominant type in a segregating family can be identified as homozygotes and heterozygotes in about the typical 1:2 ratio.

These facts show that *mut. acutifolia* is included among the small group of mutations of *Oe. Lamarckiana* which segregate in typical monohybrid ratios. This feature serves definitely to place gene a_r outside of the extensive linkage-group I, since genes in this group are prevented from appearing with the typical Mendelian frequency because of their association with the balanced lethals, 1_1 and 1_2 , characteristic of *Lamarckiana* (Shull, 1923). There are three possibilities relative to the linkage relationship of *mut. acutifolia* with other known genes: (1) either the gene for *acutifolia* is linked with *brevistylis* (b_r) in linkage-group II; (2) it is linked with *vetaurea* (v), *supplena* (s_p), and *bullata* (b_u) in linkage group III, or (3) it segregates independently from the above four genes and is thus the first recognized gene of a new linkage-group IV. The determination of the linkage relations of *mut. acutifolia* is under investigation by Dr. Shull.

The importance of finding additional genes in *Oe. Lamarckiana* can not be too strongly emphasized, for it is through these that the solution of the cytological-genetical puzzle in this species will come. As is well known from the brilliant work of the *Oenothera* cytologists, telosynapsis is quite commonly present in *Oenothera* species, and in *Oe. Lamarckiana* twelve of the chromosomes at diakinesis remain attached together and only one free pair is present. Two conflicting lines of thought have arisen in the attempt to interpret the relationship be-

tween this extensive linkage and the factor linkage as found in *Lamarckiana*.

Cleland (1929), and the majority of *Oenothera* cytologists and geneticists associate the extensive linkage-group I with the circle or chain of twelve chromosomes and postulate a theory which forms a basis for only two linkage-groups in a form with a circle of twelve chromosomes and a single pair.

Shull (1928*b*), on the other hand, believes that the chromosome circle in *Lamarckiana* need not be the basis for only one linkage-group. He does not fall into line with the essential part of the theory of Cleland and others who argue that the chromosomes are arranged in a definite order in the chain, that is, alternately maternal and paternal. Shull believes that the orientation of the chromosomes within a single homologous pair is entirely a matter of chance, but that the pairs maintain their identity in such manner that material and paternal members of the same pair are normally separated at meiosis. According to the latter view, it is quite possible to have as many linkage-groups as there are haploid chromosomes. That is, *Oe. Lamarckiana*, with its circle of twelve and one pair, could segregate for seven linkage-groups, instead of only two.

The determination of these linkage-groups will determine the answer to this cytological-genetical problem, hence the great importance to be attached to new genes which are free from the first linkage-group.

The cytology of *Oe. mut. acutifolia* has been investigated through a study of the stages of meiosis in the pollen mother cells. The $2n$ number has been found to be fourteen, as in the parent species, in accord with the genetical indications that *mut. acutifolia* represents a gene mutation. In addition, there are indications that the chromosomes of *acutifolia* in diakinesis arrange themselves in a circle or chain of twelve with one free pair. Work is now in progress to confirm this preliminary statement. With a circle of twelve and one free

pair mut. *acutifolia* will thus have the same chromosome configuration as its parent species, *Oe. Lamarckiana*.

SUMMARY

1. *Oenothera* mut. *acutifolia* appeared in the spring of 1929 in a cross-bred line of *Oe. Lamarckiana* which had been maintained for twenty-three years.

2. This new gene mutation is characterized by a narrower, more sharply pointed rosette leaf and a reduction in the amount of crinkling. Plants of *acutifolia* grow much more slowly than typical plants of *Lamarckiana* and are several weeks later, on the average, in coming to bloom.

3. When *acutifolia* first appeared it made up 21.4 per cent. of the population of three segregating families.

4. Self-fertilized *acutifolia* has bred true.

5. In a segregating family, 31 phenotypic *Lamarckiana* plants were tested and shown to be of two genetic constitutions: Eight plants (A, A_1) gave typical *Lamarckiana* progenies; twenty-three plants (A, a_1) threw segregating families.

6. *Acutifolia* is completely recessive. When it was crossed with A, A_1 *Lamarckiana*, no *acutifolia* appeared in the F_1 ; when it was crossed with *Lamarckiana* heterozygous for *acutifolia* a 1:1 segregation resulted.

7. The occurrence of typical monohybrid ratios in self-fertilized *Lamarckiana* indicates that the gene for *acutifolia* (a_1) is not in linkage-group I with the balanced zygote lethals. Either it is in group II with b_r ; or in group III with v , s_p and b_u ; or in group IV for which it would be the first known gene.

8. Mut. *acutifolia* has fourteen chromosomes ($2n$), which at diakinesis appear to be arranged in a circle or chain of twelve and a single pair. It seems, therefore, that the configuration in *acutifolia* is identical with that found in its parent species, *Oe. Lamarckiana*. The cytological work with this form is being continued.

9. The importance of new genes in *Lamarckiana* which give typical Mendelian ratios is stressed as providing the means towards solving the problem of the true significance of chromosome cohesion in *Oenothera* species.

It is a pleasure to acknowledge my indebtedness to Dr. George H. Shull, under whose guidance my work with *Oenothera* is being done, for facilities for the execution of this work, for the original pedigreed material and for his interest and helpful suggestions and criticisms which have been invaluable.

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THE EFFECTS OF INCREASING X-RAY VOLTAGES ON THE PRODUCTION OF LETHAL MUTATIONS IN *DROSOPHILA MELANOGASTER*¹

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THAT X-rays and radium induce mutations in relatively large numbers is now well established. New discoveries give rise to new problems, and the question now has become: What is the relation between the physical agent, whether X-rays or radium, and the resulting biological phenomena? More specifically, the problem concerns the effect of increased dosage on the frequency of mutation.

In work with *Drosophila* it has become apparent that the number of visible mutations occurring can be used only as a rough indication of the gene-mutation rate, as these are relatively infrequent and difficult to detect. For true analysis the more objective lethal-mutation methods, especially the *C1B* technique of Muller, have proved much more satisfactory.

Early in his studies of mutations in irradiated *Drosophila*, Muller (1928) used two dosages in which the only variable was duration of exposure. These dosages are designated in Muller's laboratory as T_2 and T_4 , the latter being twice the strength of the former. Results based on the percentages of lethals showed that "an increase in X-ray dosage causes an increased production of mutations," though Muller did not consider the numbers of these early experiments sufficiently large for an exact ratio.

Weinstein (1928) secured from the use of Muller's two dosages results substantiating Muller's own. Wein-

¹ The expenses of this investigation were supported in part by the Committee on the Effects of Radiation upon Living Organisms of the National Research Council.

stein's figures include a number of lethals and visibles which again suggest that a greater effect is obtained by means of a stronger dosage.

Hanson, also in 1928, interested particularly in the effects of the T_2 and T_4 treatments on productivity and the sex ratio in *Drosophila*, incidentally made counts of visible mutations which indicate a definite relation between their number and the dosage. His data (1928b) on the number of return mutations from bar to round indicate a similar relation.

Oliver (1930) has made the relation of the mutation rate to dosage the primary purpose of a series of experiments. From the use of five X-ray dosages he concluded that "the total number of lethals is directly proportional to the dosage used when the only factor varied is the duration of the treatment."

In all the foregoing experiments dosages were used which differed only as to the time factor. A new approach to the general problem was made by Hanson and Heys (1928, 1929), who varied the intensity of radium rays by means of a series of filters. Their results show that the percentage of lethal mutations in *Drosophila* varies directly with the thickness of the filter, and is proportional to the amount of ionization produced in air.

It is of interest to note that evidence of a relation between dosage and mutation frequency has come also from experiments with plants. Stadler (1930) used visible seedling mutations in barley as an index of the mutation rate, and found, when he varied the time of exposure of treated seeds, that the "mutation frequency increased approximately in direct proportion to dosage." He studied as well the effect of voltages within the limits 40 to 116 kilovolts. The X-rays of different wave-lengths thus produced appeared "to be about equal in power to cause mutation when applied in intensities equal in power to ionize air."

Thus a number of different factors have been investigated. On the one hand it seems reasonable to conclude

that wave-length or *quality* of X-rays has no effect on the mutation process; while other experiments give definite evidence of a relation between mutation and the dosage or *quantity* of radiation. Since dosage is the product of two factors, time and intensity, it must be considered from two angles. In the case of radium treatment, the number of mutations appears directly proportional to the intensity, while two different experiments with X-rays indicate that the number of mutations varies directly with the duration of exposure.

The present investigation was concerned with still another factor, X-ray intensity. The question in mind was this: When X-ray intensity is varied by using treatments which differ only as to the voltage across the X-ray tube, how is the mutation process affected?

EXPERIMENTAL PROCEDURE

The so-called *C1B* stock was used in these experiments. In this strain two groups of mutant, sex-linked genes are present. One X-chromosome contains the series of recessive characters, scute (*sc*), vermilion (*v*), forked (*f*) and bobbed (*bb*). The other contains the dominant *C* factor which prevents crossing-over, a recessive lethal (*l*), and the dominant gene for bar eye. One type of male and two types of females are viable. All the males have the *scvfbb* chromosome. Half the females are homozygous for these characters. The others have one *scvfbb* chromosome and one *C1B* chromosome. The first group of females shows round eyes, while the second has the characteristic heterozygous, kidney-shaped bar eye.

Heterozygous bar-eyed females were mated in pairs to wild X-rayed males. The F_1 females are bar and non-bar. The bar-eyed females were mated to their brothers, one pair in a tube, to produce the F_2 generation. The F_1 *C1B* female receives a *C1B* chromosome from her mother and a treated X-chromosome from her father. She gives to one half her sons the lethal *C1B* chromo-

some, and to the other half the treated chromosome. Due to the presence of the lethal half the sons fail to hatch. If a new lethal mutation were induced in the treated chromosome, the other half of the sons would inherit it and also fail to appear. Therefore, by examination of the F_2 cultures, it was possible to detect a lethal mutation in the X-chromosome which occurred in the male treated two generations before. To determine the number of lethal mutations it was necessary only to record the number of cultures which hatched 100 per cent. female flies.

In eleven different X-ray treatments the voltage was varied from 40 to 99 kilovolts. All other factors remained constant: tube current 5 M.A.; target distance 20 centimeters; filter, one millimeter of aluminium; and time, 28 minutes. Before and after each treatment ionization values were read from a Victoreen dosimeter. For convenience, the ionization is recorded as the reciprocal of the time in seconds which it takes an indicator to move across 1,000 divisions of the dosimeter scale. To obtain intensity values in r units per unit minute, it is necessary only to multiply by a constant k the value of which is 1,000.

EXPERIMENTAL RESULTS

The results given in Table I represent a total of twelve experiments in which eleven different dosages were used. The 95-kilovolt experiment was repeated, since the results obtained seemed inconsistent with those of the other experiments. A second trial gave data consistent with the rest.

It is seen readily from the table that both the amount of ionization and the percentage of lethal mutations increase as higher voltages are applied to the tube. Since voltage as such was not measured, the relation between voltage and mutation is of no consequence here. The essential fact for the problem in question is the correlation between mutation and ionization, which is the measure of intensity. In Fig. 1 this relation is shown

TABLE I

SHOWING THE RESULTS OF THE ELEVEN DIFFERENT DOSAGES: VOLTAGE
 VARIED AS INDICATED; 5 M.A.; 20 CENTIMETERS DISTANCE; 1 MILLI-
 METER ALUMINIUM SCREEN; TIME, 28 MINUTES. 40 KV
 DOSAGE APPROXIMATELY EQUALS 445 r UNITS;
 48 KV-675 r UNITS, ETC.

Dosage X-ray voltage	Ionization proportional to	No. fertile F ₂ cultures	No. lethal mutations	Percentage lethal mutations
40 KV	0.0159	799	12	1.502 ± 0.2902
48 KV	0.0241	746	14	1.876 ± 0.3341
52 KV	0.0285	710	13	1.831 ± 0.3394
60 KV	0.0455	607	17	2.801 ± 0.4516
67 KV	0.0588	1097	60	5.469 ± 0.4630
70 KV	0.0555	804	27	3.358 ± 0.4285
76 KV	0.0555	591	32	5.414 ± 0.6278
80 KV	0.0714	636	44	6.918 ± 0.6818
88 KV	0.0689	524	41	7.824 ± 0.7962
a) 95 KV	0.0925	770	48	6.234 ± 0.5877
b) 95 KV	0.1111	747	68	9.103 ± 0.7107
99 KV	0.1111	546	52	9.524 ± 0.8473
Control ²		1308	1	0.076 ± 0.0515

graphically. The points are plotted as lines, their limits enclosing the ranges of the probable errors. The actual points are cross-bars in the center of each line. The control percentage of mutation, 0.0764 ± 0.0515 , is taken from an experiment recently performed in this laboratory for which the same *C1B* method was employed.³

It will be noticed that one of the 95-kilovolt experiments and the 99-kilovolt experiment both gave an ionization value of 0.1111. They gave also percentages of mutation differing only by forty-two one-hundredths of one per cent., which may be considered a very small amount in such experiments as these. This agreement seems to the authors to establish rather firmly the upper limits of the curve. Now if a straight line which has its origin

² Frank Blair Hanson and Florence Heys, "A Possible Relation between Natural (Earth) Radiation and Gene Mutations," *Science*, 71: p. 44, 1930.

³ Frank Blair Hanson and Florence Heys, "A Possible Relation between Natural (Earth) Radiation and Gene Mutations," *Science*, 71: p. 44, 1930.

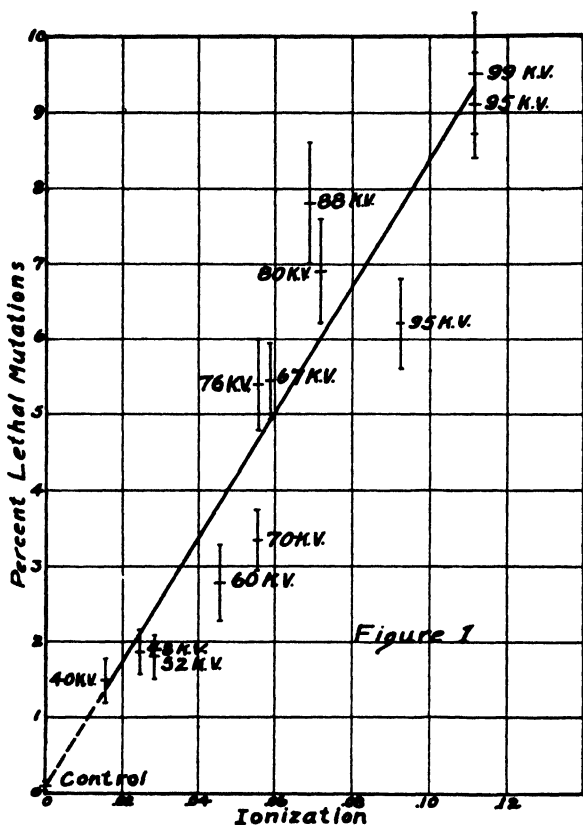


Fig. 1

between these two points is drawn so as to pass through the control point, it is apparent immediately that one half of the points fall on either side. The individual points vary rather widely from the line, but the amount of deviation in one direction is approximately balanced by that in the opposite direction. For a number of reasons this deviation is to be expected. First, the probable errors are for the most part unavoidably large. Numbers for each individual experiment can not be increased greatly beyond the point they have already reached because of the attendant difficulty in handling the flies. Second, the X-ray machine can not be considered a source of constant radiation. There may be fluctua-

tions from time to time either in current or in voltage. Over a twenty-eight minute period of treatment, these changes might be considerable.

The fact that the number of mutations occurring represents a linear function of ionization may be shown by gathering the points into groups arbitrarily, each group representing approximately 10 KV. When these results are averaged the following figures are obtained.

TABLE II

Group	Voltage in KV.	Ionization proportional to	Percentage of mutations	Point
1	40, 48, 52	0.0228	1.753	1
2	60, 67	0.0522	4.135	2
3	70, 76, 80	0.0608	5.230	3
4	88, 95a, 95b, 99	0.0959	8.171	4

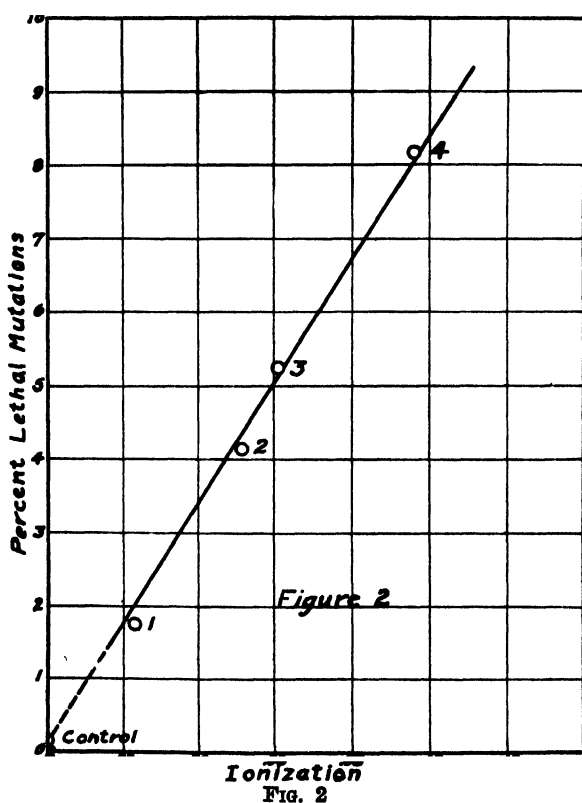


Fig. 2 shows this relationship graphically. Here the up-and-down deviations of the individual points have been smoothed out by taking the average of arbitrary groups of points which cover a short range of voltage.

DISCUSSION

From the standpoint of physics an increase in X-ray voltage is known to have two effects. First, it increases the proportion of short wave-length or *hard* rays, thereby indirectly increasing the penetrating power of the X-ray beam. Second, a rise in X-ray voltage causes a speeding up of the electrons which pass from the cathode and strike against the target of the X-ray tube. Thus the intensity of the X-ray beam is increased as higher voltages are applied to the tube.

Work by Wood (1924, 1925) on the death-rate of tumor cells, by Packard (1927, 1929) on the death-rate of *Drosophila* eggs, and by Stadler (1930) on barley mutations gives evidence that wave-length within the limits ordinarily used has no effect. Accordingly, wave-length was not taken into account in this investigation. From the results this seems justifiable, for here again, as in other experiments, no effect of wave-length is indicated.

The relative intensity was determined by methods which utilize a secondary property of X-rays, the ionization of gases. When X-rays strike matter, beta particles or electrons are knocked out from the molecules. The beta particles are moving so rapidly that they *also* may break down other molecules into pairs of ions. Most of the ionization is said to be due to this secondary beta radiation.

Two properties of X-rays have been studied in these experiments. It is known that X-rays produce ionization by means of beta particles. Since the amount of ionization is approximately proportional to the number of mutations, it seems reasonable to suppose that the beta rays which produce ionization are likewise responsible

for mutations. This theory was advanced first by Hanson and Heys (1928, 1929) whose results from experiments with radium radiation showed an almost perfect correlation between ionization and mutation. The results with X-radiation are similar though not quite so definite. The difference in the results of the X-ray and radium experiments, while not a fundamental one, is readily understood when the differences between the two sources of radiation are considered.

SUMMARY

(1) Sex-linked lethal mutations furnish good material for a quantitative analysis of the mutation process.

(2) When the intensity of the X-ray beam is increased by raising the voltage over a range of 40 to 99 kilovolts, the number of mutations is increased accordingly. Taking the amount of ionization in air as a measure, the mutation rate seems to vary approximately in direct proportion to the intensity.

(3) There is some evidence that gene mutations are due not to a number of causes, but to one, the beta particle, and that it is because X-rays and radium both produce beta radiation that they are successful agents in inducing gene mutations in the laboratory. Similarly, it is suggested that some kind of earth radiation producing beta particles may be responsible for mutant types in nature. Recent measurements by Muller and Mott-Smith (1930), however, seem to indicate that natural radioactivity is far "inadequate to explain the frequency of 'natural' mutations."

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INTERNAL FACTORS AFFECTING DISCONTINUITY BETWEEN SPECIES¹

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MAY I preface my remarks by repeating my conviction that the species problem is a problem and that as one of the fundamental problems of biology it is worthy of study as an end in itself and not as a mere corollary to work in ecology, systematics or genetics? Not until a fairly large number of species have been carefully and methodically studied over their entire range shall we be able to discuss the problem intelligently.

Aside from the work on cultivated plants by Vavilov and his associates, there are not even complete morphological surveys of any of the higher plants. For what species can the following simple questions be answered: What is its actual distribution? Does it grow in different situations in different parts of its range? What, for some easily measured character such as height or flower size, are the largest and smallest values for the species, what the average for the species as a whole? Do the values for different localities depart significantly from the mean of the species? Over what part of its range is it the most variable, over what part the least? Are there distinctive color forms and if so what is their comparative frequency in different localities? Are there any geographical regularities in the distribution of such conspicuous characters as hairiness, prostrate habit, etc., and if so, are they paralleled in other species having approximately the same distribution?

For the past six years I have been attempting to gather data which would answer just such questions. I have been studying a few species of *Iris*, the genus *Aquilegia* and several species of *Aster*. While the work is far

¹ Read at the joint discussion on "The Species Concept" at the Fifth International Botanical Congress, Cambridge, August, 1930.

from complete it has progressed far enough to convince me that species (though they are fundamental biological units) are of quite a different nature in these three genera. By this I mean that the morphological resemblances and genetic connection between the individuals which make up a species are widely different in these three groups of plants.

Lacking the precise information which would answer the question, what differences between species in these three genera may we logically expect on purely *a priori* assumptions, admitting for the purpose of the argument only that species are recognizable groups, the conclusion is unavoidable that they will, as units, be greatly affected by any factor which affects the degree of their isolation. Roughly we can classify these factors as internal and external. There must be many factors common to all *Iris*es or to all *Aquilegia*s which affect the nature and the degree of the isolation between species in each genus.

In the case of the genus *Iris* I think we may profitably consider not only that genus but practically the whole of the *Liliiflorae* (the *Iris*, *Amaryllis* and *Lily* families) since they are a natural group with many features in common. In the first place, polyploid series have been reported for a number of genera in these three families. That is to say that within many genera in this group (and sometimes even within the same species) forms occur whose chromosome numbers are simple multiple series. This will have a profound effect upon the relations between species.

To take a hypothetical example which differs in no essential detail from cases already reported in cytological literature, let XX and YY represent two diploid species, ZZZZ a tetraploid species, one letter being used to represent a haploid chromosome complement. If XX and YY, for instance, were species with twenty-four chromosomes ($2n$), ZZZZ would have forty-eight and each letter would represent a group of twelve chromosomes. XX and ZZZZ hybridizing produce a sterile

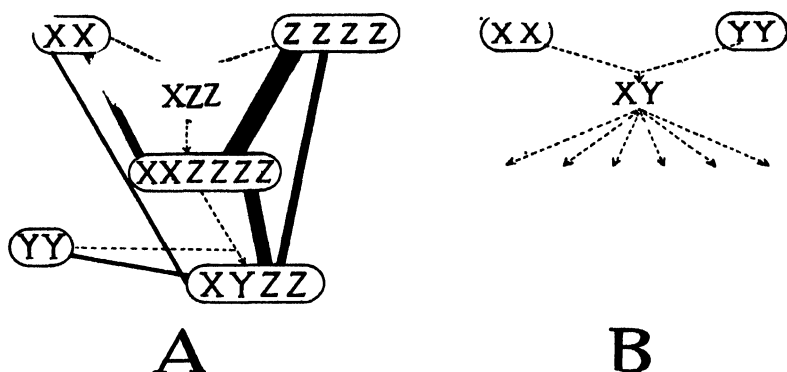


FIG. 1. Interspecific relationships in A (the Liliiflorae) and B (the genus *Aquilegia*).

triploid XZZ , which by doubling gives rise to the hexaploid $XXZZZZ$. By analogy with such cases as are known we would expect the hexaploid to be fertile and true-breeding. These relationships are represented in Fig. 1, A, the broken lines indicating the genetic relationships of the species involved.

It is so commonly assumed in monographic work that morphological resemblance is a true guide to phylogenetic origin that it may be worth while to call attention to their differences in this particular example. The broad bands indicate the morphological resemblance to be expected on the assumption that it would be proportional to the number of sets of chromosomes in common. The example is a relatively simple one. As a matter of fact much more intricate interspecific relationships might very conceivably occur. Phylogenetic relationships and morphological resemblances in such groups will be reticular rather than dendritic. That is to say that the course of evolution, instead of being represented as a tree with diverging branches, may be more aptly likened to a complex and irregular web, with threads of varying thickness.

In the second place, the Liliiflorae are distinguished by highly developed vegetative propagation, as for instance in such genera as *Tulipa*, *Lilium*, *Narcissus* and *Iris*. Hybrids so sterile that in other groups of plants they

could not survive for more than a season or two may, by vegetative propagation, live for centuries and even spread across continents. There is therefore, among the Liliiflorae, the opportunity, on the one hand, for the production of complex, intergrading forms, and, on the other, for their survival and propagation. Newton and Darlington (1929) have shown that certain common tulips are sterile polyploid clones. Many of the bearded irises (Pogoniris) are sterile, and cytological examination proves them to be complex polyploids. Hybrids between *I. virginica* and *I. versicolor* are practically sterile, yet in certain localities, by vegetative propagation, they occur to the exclusion of the parent species.

In *Aquilegia*, on the other hand, there is little or no asexual propagation. It is difficult, even in an experimental garden, to keep a particular clone alive for more than four or five years. Sterile hybrids will therefore be of no importance. There is furthermore no polyploidy in the group, either among the wild species or the numerous garden forms which have been investigated. True-breeding hybrids can not therefore be produced, and only relatively homozygous hybrid segregates will perpetuate their kind.

Fig. 1, B, continuing the symbolism of the previous example, represents the results of a species cross in *Aquilegia*. The first generation hybrid, XY, being practically fully fertile, a flood of segregating forms will be produced in the second generation which will exhibit various recombinations of the characters of species XX and XY.

An actual case of such a cross between two species of *Aquilegia* is that of *A. formosa* of the Pacific Coast of North America and *A. flavescens* of the Rocky Mountains. Payson (1918) in his detailed monograph of the North American *Aquilegias* says:

The greatest development of *flavescens* occurs in the higher mountains of Montana and adjacent Wyoming and Canada. As we go westward from this region we find the species apparently merging into *formosa*; the sepals become salmon-colored or pink, the laminae shorter, and the spurs straight.

This transitional area is often characterized by the lack of typical plants of either species, and in the mountains of Custer County, Idaho, the author has seen great patches of a variety with beautiful salmon-colored flowers entirely replacing the red *formosa* and the yellow *flavescens*. Since in the centers of their ranges *formosa* and *flavescens* are amply distinct, the author is very loath to treat one plant as a subspecies of the other. It would seem best to retain each as a species, never forgetting, however, that in certain regions the two actually merge.

There are furthermore in *Aquilegia* only slight physiological barriers to crosses between species, such as occur in practically all other genera. The most diverse Asiatic, American and European species cross readily and give fertile hybrids. Even *Aquilegia ecalcarata* Maxim. can be hybridized with *A. vulgaris*, though it is so distinct that Drummond and Hutchinson once (1920) placed it in Makino's genus *Semiaquilegia*. Species of *Aquilegia* will therefore be distinct only as long as that isolation persists. Whereas in *Iris* we may find several species growing together (as, for instance, *Iris versicolor* and *I. prismatica* in New England salt marshes) this will seldom or never occur in *Aquilegia*.

I have taken asexual propagation, polyploid series and physiological isolation as representatives of the internal factors which affect specific isolation and which whole genera or even families of plants may have in common. There must be many other such factors. May we not therefore logically expect that, even though species prove to be actual biological units, their relationships with each other and the relationships of individuals within species will vary from genus to genus and from family to family?

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SIZE GROUPS AND THEIR CHARACTERISTICS IN THE SALAMANDER *HEMIDACTYLIUM* *SCUTATUM* (SCHLEGEL)¹

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It has generally not proved possible to collect a large enough series of individuals of the four-toed salamander, *Hemidactylum scutatum* (Schlegel), for a clear definition of the age groups. Juveniles of the year, it is true, have been easy to recognize, and it is not difficult to get enough of these for a close approximation of the size limits at this age. The place to seek them is the dry, or damp, ground in the immediate vicinity of the nesting sites, as they do not wander far in the first autumn. The size limits of the adults, as well, may be fairly readily determined, although to get an adequate series of both sexes at the same time of year is not easy. The males may be had most easily in the autumn, for this is the mating season, and the females may be obtained in numbers at the nesting sites in the latter part of April and in May. Individuals intermediate in size between the yearlings and the adults may be found at all times of the year, but ordinarily very few at a time. The reasons for this are obvious. There are fewer individuals in this group than in the first-year group because there has been a year for their numbers to be thinned by such vicissitudes as enemies, accidents, weather, winter² and diseases; and the individuals are more scattered from their year of wanderings. Miscellaneous collections have given no evidence as to how many seasons this intermediate group represents.

¹ Contribution from the Zoological Laboratory of the University of Michigan.

² Mrs. Wilder has shown a heavy death-rate during the first winter for larvae of *Eurycea bislineata* (Copeia, 133: 78, 1924).

THE AGE GROUPS

One fortunate collection has now provided some real evidence on the question of age groups in this species. On the two days October 10 and 12, 1924, a series of 352 specimens of *Hemidactylum* was collected at a single locality in Hamburg township, Livingston County, Michigan, about fifteen miles north of Ann Arbor. In this collection the critical intermediate group was fairly well represented.

These specimens, as well as all others hereafter considered, were measured, freshly drowned, with dividers. For most comparisons the total length was found most useful, but the relative length of the tail has proved of value in characterizing certain groups as to age and sex. For this the tail length has been divided by the body length. The body length was taken arbitrarily as the distance from the tip of the snout to the posterior insertion of the hind leg, measured with dividers, and the tail was measured from this point to its tip. It was necessary, of course, to reject all specimens with incomplete or regenerated tails. The sex of juveniles was determined by dissection, but that of adults was often judged from the secondary sex characters.

Although we have collected *Hemidactylum* from more than twenty different places within a radius of twenty-five miles of Ann Arbor, Michigan, from only three has the species been obtained in numbers adequate to the present purpose. One of these places (Whiteoak, Ingham County) has furnished a fair series of adult females only; another (Iosco, Ingham County) has furnished only adults and yearlings, while from the other (Hamburg, Livingston County) has come the above-mentioned collection, the only one representing all ages in reasonable numbers in the same collection.

When the total lengths of the sexually immature males of this Hamburg collection are plotted by two-millimeter class intervals they form two well-defined and well-

separated groups (Fig. 1). The group containing the smallest individuals extends from 25 to 39 millimeters with a mode at about 29 to 33, and the group of larger individuals extends from 45 to 57 millimeters with an ill-defined mode at about 49 to 53 millimeters.

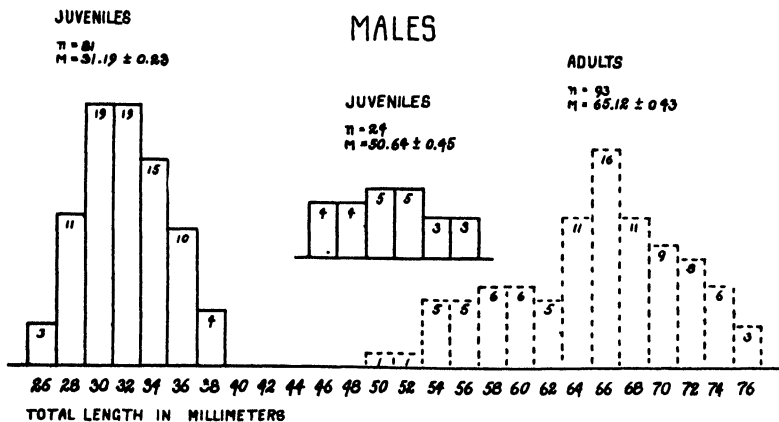


FIG. 1. Frequency polygons showing total lengths of all males collected at Hamburg, Michigan, October 10 and 12, 1924. The polygon of adult males is amplified by inclusion of 24 individuals collected at the same place on April 18 and 20, 1924. Specimens varying from 25.0 to 26.9 millimeters are plotted in the 26-millimeter group, etc. The number of specimens in each class is given near the top of each column.

The first group represents without question the individuals in their first season. The other group must be composed of individuals at least a year older. In the figure this group has been diagrammed on a short abscissa inserted above the principal one, in order not to interfere with the polygon of the adult group.

The polygon representing the distribution by total length of the sexually mature males of this collection overlaps notably that of the larger juveniles, with a conspicuous prominence well to the left of its mode at about 66 millimeters.³

³ This polygon of adult males has been amplified by the measurements of 24 adults collected at the same place on April 18 and 20, 1924. There can be no objection to this inclusion because of the slow growth of adults and the very limited growing time between October 12 and April 20 in this locality. These added figures improve the bulk of the polygon without changing its shape.

It may now be asked whether the mature males shorter than 62 millimeters are of the same age as the larger juveniles, or are one year older. If of the same age, then we have an instance of a population of uniform size at the end of the first season diverging so in development during the next year that about half become sexually mature while the other half remain juvenile. Such divergence in rate of development within one age group is not unknown. Mrs. Wilder has shown⁴ that in the salamander *Eurycea bislineata* great divergence in time of metamorphosis occurs during the second year of larval life. Transformation to the terrestrial form may take place at any time during the whole of the second season or even in the early part of the third season. Furthermore, Noble has recently pointed out⁵ much variation in the time of attainment of sexual maturity in the newt *Triturus viridescens*. Hemidactylum, however, has a briefer egg-laying season than either *Eurycea* or *Triturus* and a shorter larval period than the former at least, so that its age groups may be expected to be more clearly defined.

If, in the present case, the larger juveniles and the smaller adults are of the same age they might be expected to show an approximately normal curve when plotted together. This they fail to do, but present on the other hand a low curve of greater width than would be expected for the second-year group.

While not denying that some individuals may become mature a season earlier than their class and that some may be delayed a season longer, the writers incline to interpret the figures as indicating that the smaller males in this species are a year older than the larger juveniles. In other words, the males ordinarily attain sexual maturity in southern Michigan at the end of their third season, i.e., about two and a third years after hatching from the

⁴ The Relation of Growth to Metamorphosis in *Eurycea bislineata* (Green). Journ. Exp. Zool., vol. 40, 1924, p. 2.

⁵ Further Observations on the Life-History of the Newt, *Triturus viridescens*. Amer. Mus. Novitates, no. 348, 1929.

egg. Individuals of this age are assumed to be represented by the left-hand portion of the polygon of adults, and the modal point at 66 millimeters may well be chiefly due to males in their fourth, or a later, season.

The females collected on October 10 and 12, 1924, numbered 146, of which all but 12 were sexually immature, or non-breeding individuals. These 134 juveniles seemed clearly to belong to two size-groups (Fig. 2), separated by a well-marked interval at about 44 millimeters of total length. The group of smaller individuals is unquestionably a unit. It shows a mode at 31 to 33 millimeters and an extreme variation from 27 to 43 millimeters. The

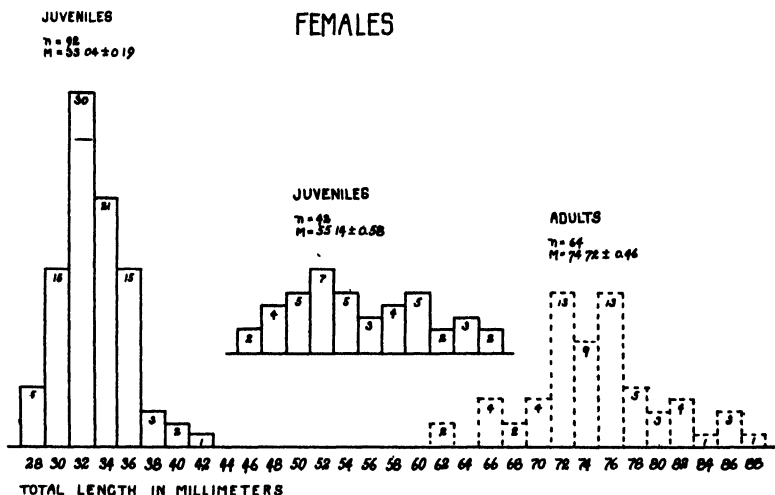


FIG. 2. Frequency polygons showing total lengths of all females collected at Hamburg, Michigan, October 10 and 12, 1924. The polygon of adult females is amplified by inclusion of 52 individuals collected at the same place on April 18 and 20, 1924. The number of specimens in each class is given at the top of each column. Specimens varying from 27.0 to 28.9 millimeters are plotted in the 28-millimeter group, etc.

other group of juveniles, composed of only half as many individuals, seems to be a compact group although it shows no clear mode. Its range of variation, 45 to 67 millimeters, is, as would be expected in an older population, a little greater than that of the younger juvenile group. One specimen 73 millimeters long, although a

non-breeder, is not included in this group, for an unusually heavy infestation of the protozoan parasite *Haptophrya michiganensis*^a led to the inference that this was an adult which had been unable to produce eggs.

Since the series of twelve sexually mature females in this collection is too small to show the position of the adult group with respect to the larger juveniles, we have added the measurements obtained from a lot of 52 adult females collected at the same place, on April 18 and 20, 1924. On account of the relatively insignificant growing season between October 12 and April 20 in this latitude and the fact that none of these 52 individuals had laid their eggs, there can be no objection to using these April specimens for determining the range in size of the breeding females.⁸ The adult group thus shown (Fig. 2) overlaps slightly the group of larger juveniles, as was noted to be the case with the male sex.

Further evidence bearing on the question of the unity of age of the intermediate group may be found in the relative length of the tail. Such evidence is more particularly to be desired for the females because the polygon formed from the total lengths of the intermediate sizes in this sex is evidently bimodal (Fig. 2). When the relative tail length of these individuals is plotted into a frequency polygon at class intervals of 0.05 a unimodal polygon of sufficiently normal form is obtained. We may from this feel safer in our interpretation that the juveniles intermediate between the yearlings and the adults belong to a single age group. A comparison of this polygon with that for the yearlings and adults is given in Fig. 3.

The frequency polygon for the male sex, based on the relative tail length (Fig. 4), looks much like that based on total length (Fig. 1), except that, as in females, there is considerable overlapping between the smaller adults and the larger juveniles.

^a Woodhead, A. E., *Haptophrya michiganensis* sp. nov. Journ. Parasit., vol. 14, 1928, pp. 177-182.

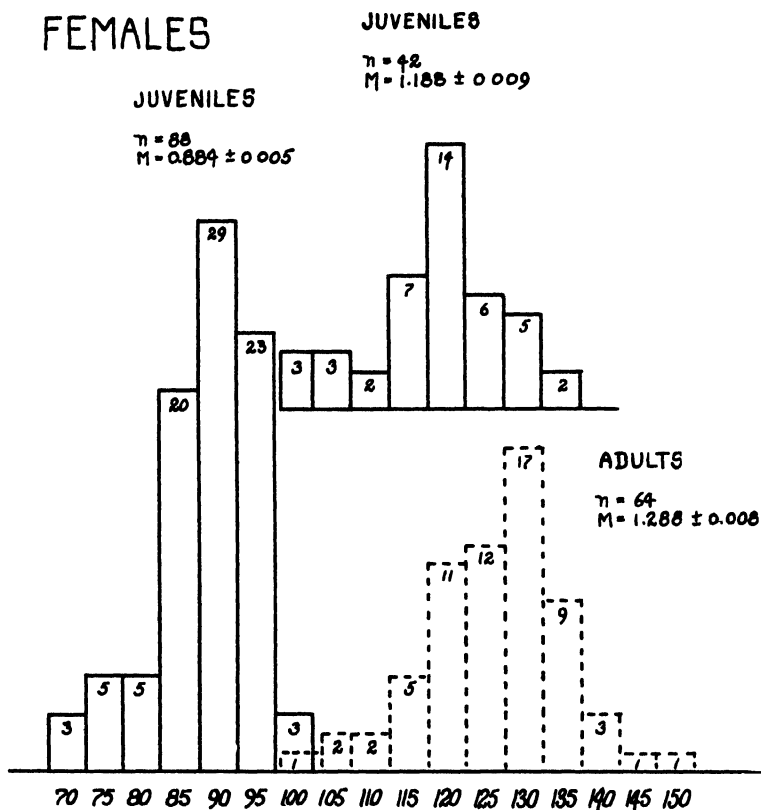


FIG. 3. Frequency polygons showing relative tail lengths (tail length divided by body length) of all females collected at Hamburg, Michigan, October 10 and 12, and, adults only, April 18 and 20, 1924. The number of individuals in each class is given at the top of each column. Specimens varying from 0.675 to 0.724 are plotted in the 0.70 class, etc.

An attempt to get direct evidence of the size of the different ages of these salamanders provided data that, so far as they go, are supportive of the conclusions derived from the measurements discussed above. A cement enclosure about 8 x 10 feet was made in a shady place and provided with a pond and woods earth and logs. Eggs from natural nests were put in moss appropriately situated above the water, in the spring of 1923. On October 23 eleven small individuals with complete tails

were recovered from the enclosure. These averaged 31.85 millimeters in total length, with a range of variation from 30.0 to 35.5 millimeters. This checks perfectly with the yearlings collected in the field at this time of year (Table II, Figs. 1 and 2).

These salamanders were revived (having been anesthetized with water for the measuring) and returned to the enclosure for another year's growth. In the spring of 1924 more eggs were put in the pond to provide a new crop of yearling salamanders for the coming autumn.

MALES.

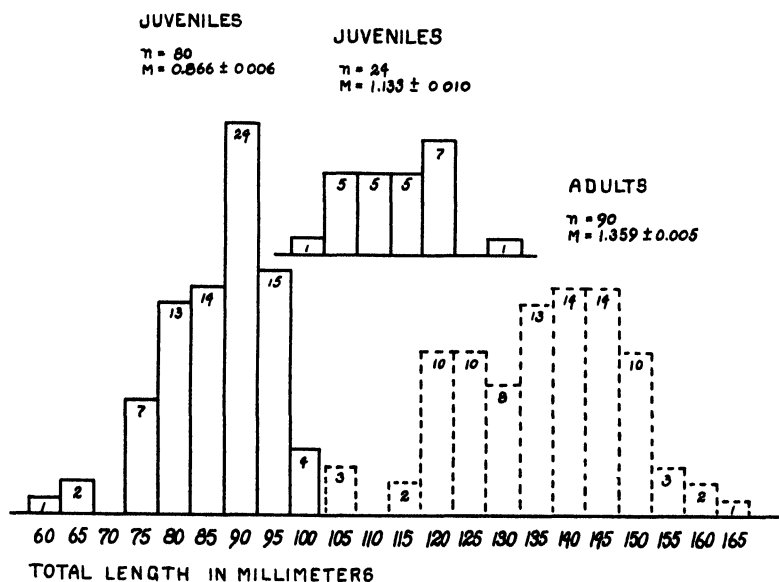


FIG. 4. Frequency polygon showing relative tail lengths (tail length divided by body length) of all males collected at Hamburg, Michigan, October 10 and 12, and, adults only, April 18 and 20, 1924. The number of individuals in each class is shown at the top of each column. Specimens varying from 0.725 to 0.774 are plotted in the 0.75 class, etc.

On October 22, 1924, the salamanders recovered from the enclosure were clearly of two size-groups, but they were all smaller than expected (Table 1). This may probably be attributed to less favorable conditions (apparently dryness), since during the ensuing season all the

salamanders disappeared from the enclosure. These two groups may, however, fairly be said to represent the two groups of juveniles that may be collected from natural habitats at this time of year. The difference between the mean total lengths (16.77 millimeters) of these two groups is only a little less than the difference between the means of the two groups of males (19.44 millimeters) collected at Hamburg at the same season of that year. In the corresponding differences between the means of the proportionate tail lengths the salamanders from the enclosure made the greater gain (0.316 in comparison with 0.265). Since a greater relative increase was made in tail length it is, perhaps, reasonable to assume that there was insufficient natural food in the enclosure to provide for normal increase in the bulkiest part of the animal, *i.e.*, the body.

TABLE I

JUVENILE SALAMANDERS COLLECTED OCTOBER 22, 1924, FROM ENCLOSURE

	Total length in millimeters	Tail divided by total length
Group of	26.0	0.852
smaller	26.5	0.783
juveniles	27.1	0.807
	28.7	0.817
	29.0	0.791
	29.3	0.773
	29.8	0.807
	$M = 28.06 \pm 0.32$	0.804 ± 0.006
Group of	42.7	1.128
larger	43.0	1.128
juveniles	44.0	1.061
	45.2	1.148
	46.2	1.162
	47.9	1.095
	$M = 44.83 \pm 0.52$	1.120 ± 0.009

CHARACTERISTICS OF THE AGE GROUPS

These salamanders in their first autumn can be recognized as yearlings by their small size alone. The largest

individual in the two principal collections made (totaling 334 specimens) was 41.6 millimeters in length, and the averages of the four groups were from 30 to 33 millimeters (Tables II and III). Between the two sexes at this age there is certainly no obvious difference in length,

TABLE II

COMPARISON OF THE SEXES AT DIFFERENT AGES IN A COLLECTION OF HEMIDACTYLUM MADE AT HAMBURG, MICHIGAN, ON OCTOBER 10, 12 AND (ADULTS ONLY) APRIL 18, 20, 1924. MEASUREMENTS ARE IN MILLIMETERS

Total length								
Age	Sex	No.	Variation		Mean	Difference between means		Difference divided by probable error
First autumn	males	81	25.8	-38.3	31.19 ± 0.23	1.85 ± 0.30		6.2
"	females	92	25.8	-41.1	33.04 ± 0.19			
Second autumn	males	24	45.3	-56.8	50.64 ± 0.45	4.50 ± 0.63		7.1
"	females	42	45.5	-66.9	55.14 ± 0.58			
Adult	males	93	49.8	-76.6	65.12 ± 0.43	9.60 ± 0.63		15.2
"	females	64	62.0	-87.7	74.72 ± 0.46			
Tail length divided by body length								
First autumn	males	80	0.612- 1.016		0.866 ± 0.006	0.018 ± 0.008		2.3
"	females	88	0.678- 1.030		0.884 ± 0.005			
Second autumn	males	24	1.012- 1.290		1.133 ± 0.010	0.055 ± 0.014		3.9
"	females	42	1.000- 1.366		1.188 ± 0.009			
Adult	males	90	1.050- 1.648		1.359 ± 0.005	0.071 ± 0.009		7.9
"	females	64	1.029- 1.484		1.288 ± 0.008			

but it is noteworthy that the females in each of the collections, Iosco and Hamburg, showed a little higher average total length than the males. In the Iosco collection this was a little over one millimeter and in the Hamburg collection nearly two millimeters. In the former case the difference in length between the sexes exceeded the probable error of the difference by three times and in the latter by a little more than six times. These small differences might not be regarded as significant if it were not for the fact that they are greatly accentuated in the adults, so it is quite possible that the females may be growing faster than the males even at this early age.

TABLE III

COMPARISON OF THE SEXES AT DIFFERENT AGES IN A COLLECTION OF HEMIDACTYLUM MADE AT IOSCO, MICHIGAN, ON NOVEMBER 9, 1924, AND (ADULTS ONLY) NOVEMBER 16, 1924, APRIL 5 AND NOVEMBER 22, 1925, AND OCTOBER 17 AND 31, 1926.

MEASUREMENTS ARE IN MILLIMETERS

<i>Total length</i>							
Age	Sex	No.	Variation	Mean	Difference between means		Difference divided by probable error
First autumn	males	76	22.1 -37.8	30.34 ± 0.29			
"	females	85	19.9 -41.6	31.55 ± 0.26	1.21	± 0.41	3.0
Adult	males	83	60.2 -87.9	73.98 ± 0.41			
"	females	41	69.7 -94.7	83.66 ± 0.54	9.68	± 0.68	14.2
<i>Tail length divided by body length</i>							
First autumn	males	76	0.675- 1.000	0.828 ± 0.006			
"	females	86	0.566- 1.071	0.831 ± 0.007	0.003	± 0.009	0.3
Adult	males	81	1.173- 1.844	1.490 ± 0.009			
"	females	42	1.173- 1.600	1.377 ± 0.011	0.113	± 0.014	8.1

Another feature of the yearling group is the notably short tail, a character that can be readily appreciated with the specimens in hand. In salamanders of this age the tail is a little shorter than the body, while in those a year or more older it is nearly always a little longer, unless injured or regenerated. Regenerated tails can usually be recognized by their slightly different appearance from normal tails. They are apt to be a little shorter and thicker and to have less sharply defined black markings on the under side. The mean tail length divided by the body length varied in the two collections of yearlings from 0.83 to 0.88. Although these figures were slightly higher in the females in each case, this difference may be regarded as insignificant from the fact that it exceeded the probable error of the difference less than three times (0.3 and 2.3), and also from the fact that in adults this proportion is markedly greater in the other (male) sex.

Individuals comprising the group intermediate between the yearlings and the adults (and here regarded as

at the end of their second season of life) are separated from the yearlings on total length measurements by a distinct gap, but overlap distinctly with the smaller adults. Only one collection was obtained in which this group was at all adequately represented. In this the mean of 24 males was 50.6 millimeters and of 42 females 55.1 millimeters. The difference, which is further accentuated in the adults, exceeded its probable error about seven times (Table II).

The tail length divided by the body length in the salamanders at the end of the second season varies from 1.00 to 1.37, with means of 1.13 and 1.19 for the males and females, respectively. The slight difference between the sexes exceeds its probable error by 3.9 times. This difference may be regarded as of doubtful significance since in adults it is the males that have the longer tails.

As in the yearling group, individuals of the second season are not externally distinguishable as to sex.

The adults are readily recognizable as such in autumn and early spring, but after the eggs are laid the smaller females may not be distinguishable from the larger juveniles. The measurements on which the following comparisons are based were taken from adults collected at various dates from October 10 to the end of the season (usually about one more month in this vicinity) and in early April, before the eggs were laid. The collections were made at Hamburg and Iosco. The variation in the total length of the males was from 50 to 88 millimeters with averages of 74 and 65 in the two collections (Tables II and III). In the females there was variation from 62 to 95 millimeters, with averages of about 84 and 75 in the two lots (Tables II and III). The average difference between the sexes was the same in each case. The females were $9\frac{1}{2}$ millimeters longer than the males (Tables II and III).

The relative length of the tail in the adults, although on the average distinctly greater than in the two-year-old individuals, practically wholly overlaps the figures for

this latter group. The variation is from 1.03 to 1.84. The averages are from 1.28 to 1.49. The difference between the sexes was 0.07 in one collection and 0.11 in the other, differences that exceed their probable errors by about eight times (Tables II and III).

The adult male may be recognized by its more truncate snout with larger swellings in the regions of the naso-

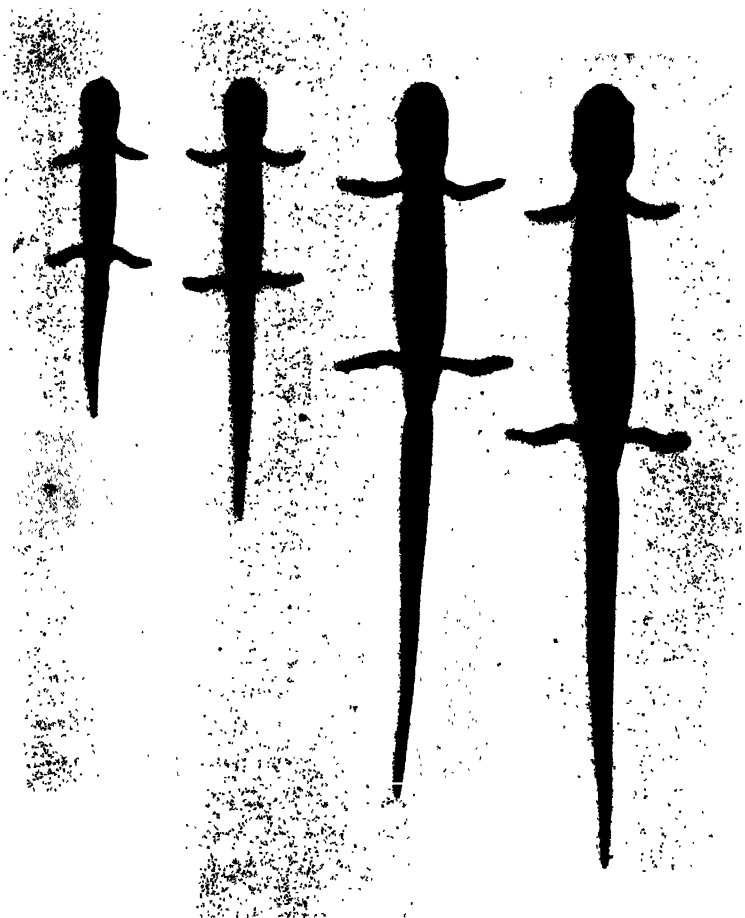


FIG. 5. Photograph of individuals of *Hemidactylium scutatum* representing the three size groups in autumn. Left to right: juvenile in its first autumn, juvenile in its second autumn, adult male, adult female.

labial grooves and by the smaller amount of pigment in this region than in the female, as well as by its shorter and more slender body and longer tail.

The relative sizes and proportions of individuals representing the three groups are shown in Fig. 5. The two salamanders at the left are juveniles of the first and second autumns, respectively; the two at the right are adult male and female individuals, respectively.

VARIATION OF SIZE WITH HABITAT

Unexpectedly it was observed during the compilation of these figures that the adults from the Iosco habitat were decidedly larger than those from the Hamburg collecting place. The males as well as the females from Iosco averaged 9 millimeters longer than the respective sexes from Hamburg, differences exceeding their probable errors by 12 to 15 times (Table IV). The proportionate length of the tail varied in the same way. The differences between the means for the two localities exceeded their probable errors by $6\frac{1}{2}$ times in the females and 13 times in the males.

Although the young salamanders in their first season showed differences, in these measurements, between the same sex in the different places, these differences were relatively slight, and furthermore, they were reversed, *i.e.*, the salamanders from the Hamburg locality were the larger.

One other collection, that of 46 adult females from Whiteoak, has a bearing on this problem of variation with habitat. The mean of the total lengths in this collection was practically identical with that of the adult females from Hamburg, differing by only 0.6 of a millimeter; and the proportionate tail length differed but slightly more.

These figures are too few to prove anything but they suggest the possibility of significant variation between habitats. It would be mere speculation to try to account for the marked similarity between the adult females from

TABLE IV
COMPARISON OF LIKE SEXES AND AGES OF *HEMIDACTYLUM* FROM DIFFERENT HABITATS

Total length								
Place	Age	Sex	No.	Mean	Difference between means		Difference divided by probable error	
Hamburg	1st autumn	male	81	31.19 ± 0.23				
Iosco	"	"	76	30.34 ± 0.29	0.85	± 0.30	2.8	
Hamburg	1st autumn	female	92	33.04 ± 0.19				
Iosco	"	"	85	31.55 ± 0.26	1.49	± 0.32	4.7	
Hamburg	adult	male	93	65.12 ± 0.43				
Iosco	"	"	83	73.98 ± 0.41	8.86	± 0.59	15.0	
Whiteoak	adult	female	46	74.09 ± 0.62				
Hamburg	"	"	64	74.72 ± 0.46	0.63	± 0.77	0.8	
Hamburg	"	"	64	74.72 ± 0.46				
Iosco	"	"	41	83.66 ± 0.54	8.94	± 0.71	12.6	
Tail length divided by body length								
Hamburg	1st autumn	male	80	0.866 ± 0.006				
Iosco	"	"	76	0.828 ± 0.006	0.038	± 0.0085	4.5	
Hamburg	1st autumn	female	88	0.884 ± 0.005				
Iosco	"	"	86	0.831 ± 0.007	0.053	± 0.0086	6.2	
Hamburg	adult	male	90	1.359 ± 0.005				
Iosco	"	"	81	1.490 ± 0.009	0.131	± 0.010	13.1	
Whiteoak	adult	female	44	1.251 ± 0.011				
Hamburg	"	"	64	1.288 ± 0.008	0.037	± 0.014	2.6	
Hamburg	"	"	64	1.288 ± 0.008				
Iosco	"	"	42	1.377 ± 0.011	0.089	± 0.014	6.4	

Whiteoak and Hamburg and their marked difference from those of Iosco. We will merely observe, as having a possible bearing, that the Hamburg and Whiteoak localities are relatively limited and isolated and that the Iosco place is part of an extensive swamp.

SUMMARY

1. Evidence has been presented in favor of the view that *Hemidactylum* attains sexual maturity near the end of its third season of life, i.e., when it is approximately two and a third years old.

2. Juveniles in their first autumn can be recognized by their small size (about 26 to 41 millimeters of total length) and by their short tails (less than the length of the body measured to the posterior insertion of the hind leg). There is practically no difference in length between the sexes at this age, but the mean for the females is slightly, perhaps significantly, greater than that for the males.

3. Juveniles in their second autumn are distinctly longer than the yearlings, but as a group they overlap in length the shorter portion of the adult group. The females average about four millimeters longer than the males. The relative length of the tail is not significantly different between the sexes at this age. It is distinctly different, however, from that of the yearlings, for it is nearly always longer than the body.

4. The adults are, of course, larger than the juveniles, except for some overlapping. The females are longer by $9\frac{1}{2}$ millimeters, on the average, than the males, and, when bearing eggs, have distinctly wider abdomens. The males have relatively longer tails than the females, and are recognizable otherwise by differences in the shape of the head.

5. There is some evidence that differences in local conditions may have a decided influence on the sizes attained by the different populations.

HYBRID VIGOR—A FACTOR IN TETTIGID PARTHENOGENESIS¹

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It has been shown by Nabours (1919, 1925, 1929), Nabours and Snyder (1928) and Nabours and Foster (1929) that heterozygous partheno-reproducing Tettigidae give segregates, practically all females, that are with rare exceptions homozygous. Furthermore, such females when allowed to mate produce offspring that show the characters of both parents as well as some that show only matroclinous characters (*loc. cit.*). Robertson (1930a) has shown that partheno-produced individuals are diploid and also that in their somatic and gonial cells like chromosomes have a tendency to lie together. The discovery of Nabours and observations of Robertson suggest that the partheno-produced tettigid arises from an egg that has given off but one polar body. Also, the breeding results (Nabours, 1919, etc.) show that the mechanism must be one that provides simultaneously for parthenogenetic and biparental reproduction. It may be supposed that the egg normally gives off the first polar body and waits for the sperm to enter. In the absence of the sperm, after some delay, the eggs of most females may degenerate, but eggs of some females evidently have the capacity to proceed with development without the second maturation having taken place.

That apogamy and parthenogenesis are in some way connected with hybridization was first suggested by Ernst (1917, 1918), as a result of his studies on the stone-worts. He noted that in varieties exhibiting such methods of reproduction the vigor and general character of the plants, the numbers of chromosomes and their irregu-

¹ Contribution No. 122, department of zoology, Kansas Agricultural Experiment Station, Manhattan.

larities during the meiotic divisions and the absence of such irregularities in bisexual varieties were suggestive of hybrids. These conclusions were supported by a number of other plant workers (Rosenberg, 1917; Winge, 1917; Holmgren, 1919; Täckholm, 1920, 1922; *et al.*). Harrison and Peacock (1925) in their studies on moth crosses came to the conclusion that hybridity was a factor in bringing about parthenogenesis. They mentioned heterosis but did not emphasize it. When the work of Nabours (1919, 1925) on *Apotettix* came to their notice they saw that he had brought together colonies from different regions and suggested that in the Tettigidae also there might be a "case of parthenogenesis consequent upon hybridity" (Peacock and Harrison, 1926). Nabours (1929) and Nabours and Foster (1929) accepted this as a possible cause but added the fertile suggestion that parthenogenesis might have come about through the bringing together of complementary factors that had previously been segregated in regional varieties.

Whatever the cause of hybrid vigor may ultimately be found to be, whether a matter of stimulation, a presence of a combination of dominant size factors, heterozygosis itself as such, a series of linked or unlinked growth factors, or in some cases homozygous hybridity (recombinational from species' crosses), it would seem that all theories should agree in this: that it is brought about by hybridization, and that it may express or manifest itself in such ways as rapidity of growth, increased ultimate size, increased fecundity and the like. These phenomena in turn involve increase in size of the cell and with it increased rate of cell division. Hybrid vigor may therefore be looked upon possibly as having a speeding-up effect on cell division and of course among other things upon the growth and resultant division of the chromosomes.

It seems reasonable then to suppose that hybrid vigor might be expressed in the oocyte and egg of these tettigid hybrids in such a way as to advance the growth and con-

sequent splitting processes in the chromatin threads during synapsis and succeeding stages to the extent that after the first polar body, *e.g.*, is thrown off the chromatids of the diads may each have received an impetus sufficient to cause them to continue these processes during the supposed period of the pause for the entrance of the sperm. As a result of this they may be supposed to have proceeded far enough in their division to form the first cleavage nucleus, in this way starting the development of the embryo. Hybrid vigor, through advancing the condition of the chromosomes, might therefore possibly serve as the stimulus to push the delaying egg over into parthenogenetic development.

However, if hybrid vigor be accepted as a factor bringing about parthenogenesis in the Tettigidae, and it be assumed that this vigor is due to heterozygosity, the following fact looms in opposition. The F_1 individual which has the capacity for parthenogenesis is a hybrid, but Nabours (1925, 1929) has found the F_2 (with rare exceptions) and succeeding parthenogenetic generations to be homozygous for their characters and yet parthenogenesis to continue. Obviously the hybrid vigor push should be absent in the eggs of F_2 and later generation females unless the vigor stimulus be some substance in the cytoplasm that is handed on in gradually diminishing amount. But there is a possibility that the F_2 and later generation parthenotes² may not be homozygous for all their characters. As has been shown by Robertson (1930a) one maturation division, and that reductional in the main, would take care of segregation such as is shown by the breeding of heterozygous parthenoreproducing females, and the equational division if omitted or if it becomes the first cleavage mitosis would agree with the occurrence of homozygosity in the segre-

² The term *parthenote* is here proposed for the designation of an individual that has developed from an egg containing a single pronucleus, and should correspond in its usage to the term *zygote* which designates an organism that has arisen from an egg in which there were two pronuclei.

gates and their descendants. It has been known for some time that in the Tettigidae homologous chromosomes are split before they pair in parasynapsis (Robertson, 1916, 1917, 1919, 1930b). The same had been discovered by Bridges (1916) in *Drosophila* from genetic data. If this be well established for the Tettigidae then it would be expected that, in addition to four-strand crossovers at one locus, there should occasionally be simultaneously a two-strand crossover at a different locus, thus resulting in four different strands to be distributed at the two maturation divisions. If this be the case, that it may be suspected will be shown later, when more distantly linked genes are discovered (*e.g.*, the θ character in item 41, Table II, and mating 229, Table V, in Nabours and Foster [1929] for *Paratettix*), one maturation division would not suffice. In *Apotettix* among the twelve different factors reported for five different loci (Nabours, 1925) the highest average crossing-over percentage between the genes at the extreme ends of the linkage group is 7.43. This would indicate a rather close linkage and probably a very small proportion of the length of a chromosome to be involved. If such be shown to be the case, and it be accepted as proved cytologically that the second (so-called equational) division is omitted in parthenogenesis (Robertson, 1930a), it would be expected that some of the F_2 segregates should exhibit, with respect to some of their characters, either a uniform hybrid condition, or a mosaic condition (two types of homozygous parts) with reference to the right and left or anterior and posterior portions of the body. This would depend of course upon whether the first cleavage mitosis occurs along the split between chromatids, that should have taken place at the second maturation, or whether it occurs along the real equational split in each chromatid itself. In one case the soma would exhibit a uniform heterozygous condition and the germ-cells would show both segregation and crossing over. In the other case the soma might be ex-

pected to exhibit, so far as this particular character is concerned, mosaics, and the germ-cells might be of two sorts (each homozygous) exhibiting cellular segregation but no crossing-over phenomena.

From the cytological evidence therefore and from the fact that all the factors so far considered are in such a short linkage group the conclusion would not be warranted that the F_2 parthenogenetic segregates should be homozygous for all their genetic factors. They might be so for the short linkage group which was being dealt with in *Apotettix* (Nabours, 1925), but for those factors that should have been segregated in the second maturation division, that evidently did not take place, they should be heterozygous. If both maturation divisions are concerned in segregation, possibly there might be differing amounts of segregation in each division, the first effecting the major part of it, the second the minor part. F_2 parthenotes should then for the majority of their genes be homozygous segregates, but to a less extent for other characters they should be heterozygous. Through succeeding parthenogenetic generations the offspring should gradually become homozygous for all their characters. If hybrid vigor is bound up with heterozygosity then parthenogenesis, in case it be induced by hybrid vigor, should gradually disappear. On the other hand, if parthenogenesis be due to the bringing together of complementary factors (Nabours, 1929) a basis would be afforded for a permanent (homozygous) condition of heterosis (hybrid vigor), and with it the possibility of continued parthenogenesis. The breeding results should decide between these suggestions, and possibly throw light upon the cause of hybrid vigor itself. First of all it should be established cytologically that but one polar body is thrown off (we are fairly certain of this now [Robertson, 1930a]). In the second place it should be established genetically whether the F_2 and later generations of parthenotes are totally homozygous (the distantly linked gene, θ , in *Paratettix* seems

to indicate they are not [Nabours and Foster, 1929]), or in part heterozygous, and in the third place whether parthenogenesis in the Tettigidae may be continued indefinitely (it has been continued for seven generations in *Apotettix* [Nabours, 1929]).

The hybrid vigor hypothesis might be applied to some cases of alternation of parthenogenetic with biparental reproduction such as occurs in rotifers, aphids and *Hymenoptera*. Whitney (1912) and Shull (1912) found in rotifers that continued parthenogenesis resulted in loss of vigor, finally ending in the production of a bisexual generation which in turn started anew the parthenogenetic portion of the cycle. In the Tettigidae parthenogenesis and alternation seem to be in an incipient condition. In the rotifers and the aphids both types of reproduction and their alternation are well established, but the parthenogenetic portion of the cycle seems to be indefinite and capable of being prolonged. This seems to argue against hybrid vigor of the heterozygosity type as being a factor bringing about parthenogenesis, although it has been established that the second polar division does not take place (Whitney, 1909), and of course the possibility remains that the parthenogenetic lines may in the early generations at least be heterozygous. In the majority of the *Hymenoptera* the alternation seems fixed and is limited to one bisexual generation alternating with one parthenogenetic generation. Is it possible that hybrid vigor, as a cause of parthenogenesis, induced by wide crosses in the Tettigidae has become incorporated in ordinary bisexual reproduction within the variety in (the rotifers and) *Hymenoptera*? Are we certain that the male in the bee is haploid? Is it possible that he might be diploid and the female tetraploid? The "coupling" (Nachtsheim, 1913) of like chromosomes in the second spermatocytes and somatic cells of the male and in the oogonial and somatic cells of the female may in some way be a case of the association

of homologues similar to that which occurs in the partheno-produced Tettigidae (Robertson, 1930a). Is there a possibility of combining these suggestions with that of Nabours' (1929) complementary factor hypothesis (which essentially means homozygous for heterosis-producing factors) in an explanation of the alternation of biparental with parthenogenetic reproduction as it occurs in this group?

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SEX DIMORPHISM AND SCHOOLING BEHAVIOR AMONG FISHES

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IN a previous paper¹ the writer has attempted to show how the psychological mechanism involved in the aggregation and maneuverings of individual fishes in schools may probably be explained as composed of a very simple set of purely automatic reactions. Greater difficulties are met with in an endeavor to explain the phylogenetic or ontogenetic origin of these reactions. It is not *a priori* inconceivable that they should properly be regarded as manifestations of ontogenetically acquired habits, but their early appearance in the life of the individuals and their more or less fixed relationship to definite systematic groups of fishes indicate the probability of their being based upon the phylogenetically developed, inheritable, nervous and morphological organization of the species after the manner of tropisms and sex instincts.

With our present inadequate knowledge of the physical background for instincts and nervous reactions, it is impossible to attempt any direct explanation for the evolution of the schooling "complex," unless the theory of phylogenetic adaptation according to the requirements of the organism be dogmatically adopted as a necessary axiom of biology. But, granting the evolution of the schooling instinct as an inexplicable quality in the nervous organization of many fishes, we may be able to determine some of the factors influencing its varying degrees of manifestation in the different systematic or biological groups, by studying the relation-

¹ A. E. Parr, "A Contribution to the Theoretical Analysis of the Schooling Behavior of Fishes," Occasional Papers, Bingham Oceanographic Coll. No. 1, 1927.

ship of the schooling behavior to the entire habit patterns of the various forms.

In the case of the inherent reactions of any organism the simple elements must be considered in their coordination with each other and their subordination to the whole to a far greater extent than in the study of the merely structural morphology, as almost any one of the many instincts centered around the various stimulations to which the organism is able to respond may temporarily or permanently serve to suppress the manifestations of any or all of the other complexes in the nervous organization of the species, or may equally serve to increase their external effect. It will be sufficient to mention the complete suppression of even such a fundamental psychological factor as the feeding instinct during the breeding season of many fishes. Without in any way intending to attribute to the fishes a capacity for consciously harboring complexes of "suppressed desires," the writer simply wants to call attention to the fact that certain normal, even vital, inherent reactions may partly or completely disappear for a considerable length of time during the life of the individual fish, while the neurophysiological mechanism of the instinct involved apparently remains intact and ready to respond as soon as the inhibiting complex returns to a passive state. In discussing the occurrence and varying development of the schooling behavior of the different forms we must therefore not only observe the external manifestations of the schooling instinct, but we must also consider the possibility of these manifestations having been altered, promoted, reduced or even entirely suppressed by the interference of other compatible or incompatible instincts in the psychological make-up of the species considered. Our task is, then, to try to make out the possible inhibiting or promoting factors in the established habit-patterns of recent fishes, thereby trying to find an explanation for the occurrence and development of their

schooling performances as such, without considering the origin of their schooling instinct in itself.

Among the factors which may possibly be of importance in this respect it is natural first to consider the sexual complexes, as these are the only, or at least by far the most important, elements in the nervous organization agreeing with the schooling complex in having for their adequate stimulus the perception of other individuals of the same species. A comparatively strong interference by the corresponding responses should therefore be expected to develop.

In the ideal school, which is very closely approached by the actual schools of such forms as the herrings or mackerels, the individual fishes are equidistantly adjusted to parallel directions of swimming, according to their mutual attraction for each other, in the manner already discussed and explained by the author (*loc. cit.*). In a school of this kind the sexes are freely mixed without any differentiation in their behavior or spatial arrangement. If now a differential sex attraction develops, it is obvious that the uniform aggregation of schools will no longer be insured by a homogenous stimulation of all individuals in a given population. Points of weakness will develop between fishes of the same sex and points of comparatively strong attraction between opposite sexes. Uniform schooling can not even under these circumstances be regarded as *a priori* impossible, but it is practically inconceivable that schools of any considerable size or uniformity should be able to aggregate and remain together under such conditions, while exposed to the constantly disturbing and disrupting influences of their natural environment. Any aggregation of individuals would rapidly break up into smaller units or pairs of a heterosexual nature. But, for a differential sex attraction to develop, it is necessary that there should be a perceptible difference between the sexes. Very little is known about the adequate stimuli for the sexual attraction among fishes, but it is logical to assume that

they should in most cases be of the same sensory quality as the stimuli for the analogous, but asexual, schooling attraction. The writer has already previously (*loc. cit.*) by experiment and general considerations attempted to show that the common and most perfectly harmonious type of schooling performances is probably entirely based upon visual perceptions alone, and we will therefore in the following discussions assume the visual perceptions to be also, in general, the causes of sexual attraction among fishes, testing the justification of our assumption by the validity of the conclusions it will lead us to. Our theoretical condition for a differential sex attraction to develop is, then, that there must be an externally visible difference between the sexes. If such differences are not present, the reacting individual is without means of discriminating the opposite sex from its own, and consequently can not be more strongly stimulated by one than by the other. What peculiarities the nervous organization of a species may develop under such circumstances is a matter of conjecture, but it seems obvious to the writer that the mechanism of sex attraction, if not entirely replaced by an already developed schooling instinct, can not become lost without leading to the immediate extinction of the species for lack of adequate propagation. It is rather to be expected that the mechanism must, in the surviving forms, have changed from heterosexual to an ambisexual orientation, causing attraction towards all individuals of the same species irrespective of their sex. The sexual complex itself may in other words be supposed to have changed into an instinct for indiscriminate schooling behavior.

Comparing these theoretical conclusions with the actual observations we find that the groups showing the most perfectly harmonious schooling performances, such as herrings and mackerels, are conspicuously lacking in external sex differentiation, and also do their spawning in indiscriminate schools, apparently in most cases without any indication of a heterosexual orientation. Even

the expert with years of experience in the study of the common *Clupea harengus* is unable to determine its sex without examining its gonads or genital products. Our knowledge about the occurrence of sex dimorphism among fishes is unfortunately rather scanty, but where adequate information is available, we find as a general rule that schooling behavior is not observed in conjunction with conspicuous external sex differentiation. The case is particularly clearly illustrated by the fauna of the tropical coral reefs where the adult representatives of the strikingly dimorphous families *Labridae* and *Scaridae* have never been observed by the writer to exhibit any indication of a schooling behavior, while the monomorphous *Haemulidae* are usually moving around in the same environment in indiscriminate schools of varying sizes. Similar evidence is contributed by the fact that schooling performances are far more commonly found among the immature than among the mature fishes.

The list of indiscriminately and more perfectly schooling types, such as the herrings and mackerels, can, on the other hand, be increased by numerous species among the less conspicuously differentiated or absolutely monomorphous forms of such families as the *Gadidae*, *Atherinidae*, *Mugilidae*, *Belonidae*, *Scomberesocidae*, *Hemiramphidae*, *Exocoetidae*, *Carangidae* and *Gerridae*, and by several *Cyprinidae*, *Salmonidae* and *Sciaenidae*.

Even more interesting evidence is contributed by the only periodically dimorphous forms, such as the common European stickleback, *Gasterosteus aculeatus*, whose males, during the breeding season, develop a very brilliant red color on the otherwise silvery belly. Concomitantly with the appearance of this character, the rather perfect schools of the species in question rapidly disintegrate and the social and indiscriminate schooling instinct gives way to a very pronounced and pugnacious individualism with a heterosexual orientation towards the former companions. After the spawning season is

over the bright coloration disappears and indiscriminate and very harmonious schools are again observed.²

Parallel conditions also seem indicated in the seasonal differences in the schooling behavior of various freshwater fishes observed by Evermann and Clark³ and particularly clearly described in the case of *Pimephales notatus* concerning which these authors report (*loc. cit.*, p. 343) that the specimens are usually found "singly or a few together" during the spring and summer, "but later they bunch up and in the fall and winter they are found in considerable schools," the scattering thus centering around the breeding season of the species (given as June). Similar observations are also recorded for *Notropis blennius* (*loc. cit.*, p. 351) and for *Fundulus diaphanus* (p. 371). In their breeding habits these species, like the stickleback, show a temporary ability to react heterosexually.

Evidence of a similar correlation between sex attraction and schooling behavior in perfectly monomorphic forms may possibly also be borrowed from A. C. Johansen's⁴ explanation of the regular, annual disappearances staged by most of the European races of herring (*Clupea harengus*) as being due to an extensive scattering mainly of the adult fish, when, "as spents or recovering spents, they go hunting for food in a more pronounced degree than otherwise." While there is obvious reason to believe, with Johansen, that the feeding instinct would

² This description of the seasonal changes in the social orientation of *Gasterosteus aculeatus* is based upon many years' observation of this species in European waters. The fact that the stickleback, outside of its spawning season, is found in typical, indiscriminate schools is only occasionally referred to in the literature (*vide* C. V. Otterström, "Fisk. I. Pigfinnelisk," p. 114-115, in "Danmarks Fauna," Copenhagen, 1912), most authors being too preoccupied with the fascinating breeding habits of this interesting little fish to remark upon its behavior during the rest of the year.

³ B. W. Evermann and H. W. Clark, "Lake Maxinkuckee," Dept. of Conservation, State of Indiana, 1920.

⁴ A. C. Johansen, "On the Migrations of the Herring." Concluding remarks, p. 27, in *Journal du Conseil* (Perm. Internat. Expl. de la Mer), Vol. II, No. 1, Copenhagen, 1927.

manifest itself with particular strength shortly after the breeding season ended, it must, on the other hand, also be remembered that this period of assumed scattering would also be coincident with the period of the probably greatest reduction of all manifestations of sexual attraction, which may otherwise lend their support to the maintenance of a schooling behavior, in the absence of externally perceptible sex differentiations in this species.

It might seem from these considerations as though indiscriminate schooling behavior and differential sex orientation could be perfectly equivalent responses of the identical psychological mechanism to the varying conditions of sexual di- or mono-morphism. Certain observations, however, while substantially confirming the relationship between schooling behavior and sex attraction as above made out, at the same time clearly tend to show that there must also be an independent complex for each of the two phenomena. It would, as already indicated in the above-discussed example of the herring, be quite unnatural and entirely discordant with all our knowledge about such matters to assume that the mechanism of sexual attraction should be equally well developed at all stages in the life of the individual fish, or that it should always be equally active after the mature stage has been reached. We nevertheless find the schooling performance much more frequent among young fishes than among their adults, as already above mentioned. We further observe how, for instance, the Cyprinodont, *Fundulus diaphanus*, as described by Evermann and Clark (see above), in spite of permanent and conspicuous sex dimorphism, is yet habitually living in large schools, which, however, show a distinct tendency toward partial or complete disintegration into smaller schools or single pairs during the spawning season, when the sexual instinct becomes active. On the basis of such observations of increased schooling during periods of presumably undeveloped or less active sexual instincts it therefore seems logical to assume that the schooling instinct as

such must be a separate element of a probably very wide distribution in the nervous organization of fishes, while the occurrence of its external manifestations in the form of actual schooling behavior is largely dependent upon and explainable by the promoting or suppressing influence from the sexual orientation of the individual, according to the varying degrees of externally perceptible sex differentiation.

It must, however, not be overlooked that numerous other factors also must be expected to exert a great, if not so general, influence upon the schooling performances of the individual species. The fact that a pelagic habitat seems more conducive to schooling than does a benthonic mode of life has already previously been mentioned by the writer, and the survival value of social or solitary tendencies according to the entire habit-pattern of the various forms, their feeding, locomotion, etc., is a factor of obvious importance in the single cases.

THE LONGEVITY OF ENCYSTED COLPODAS

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IN 1929 Dawson and Mitchell reported the recovery of a number of motile organisms from Cuban hay which was gathered by the senior author on May 1, 1925. These included a variety of micro-organisms, *e.g.*, *Colpoda cucullus*, *Oxytricha* sp., *Spathidium spathula*, *Podophrya fixa*, *Physarum cinereum* and nematodes of the genus *Cephalobus*. Cultures made in May, 1929, yielded only the following: *Colpoda*, *Oxytricha*, *Spathidium*, *Physarum* and *Cephalobus*. In this earlier paper the literature concerning the longevity of infusorian cysts was reviewed and need not be referred to here.

A series of cultures was made beginning August 21, 1930, to ascertain which of these organisms could still be recovered from the encysted state after a period of approximately five years and four months. Infusions of the dry hay were made using glass distilled water and sterile Tashmoo spring water. For the cultures with the larger quantities of medium, finger-bowls were used; for the smaller quantities, Syracuse watch-glasses. The experiments were conducted at room temperature which, during the period, varied from 22° to 30° C. In all but one of the infusions made living motile organisms were recovered. All cultures were examined at regular short intervals after they were begun (with the exception of one or two of the Syracuse watch-glass cultures) for the purpose of recording the first appearance of the excysted organisms. The record of the different infusions made is summarized as follows:

Controls were carried in each series. In none of these were any organisms seen. A study of the record of the various series shows that motile colpodas were recovered from each of the four types of infusions and active nema-

SERIES I

Number	Date	Medium	Amount of medium	Weight of hay	First observation of animals (in hours after starting culture)	Number seen at this time	Name	Remarks
I	Aug. 21	Glass distilled water	100 cc	1.9 g	24½	6	<i>Colpoda cucullus</i>	Number of colpodas increased up to about 8 days and then slowly declined.
II	“ “	“	“	.9 g	41	14	* { <i>Cephalobus elongatus</i> sp. <i>Aphelenchus</i> sp. <i>Rhabditis</i> sp. <i>Colpoda cucullus</i>	Similar to I except the number of colpodas was slightly less at any time.
					24½	3		
					41	10		
III	Sept. 2	“	“	1.5 g	264		Nematodes as above <i>Ameba</i> sp.	The anebae observed were a small species resembling <i>A. bigemma</i> and were present at this time in considerable numbers.
					18½	5	<i>Colpoda cucullus</i>	
IV V	“ “ “	“ “ “	9 cc 9 cc	.13 g .07 g	120	few	Nematodes as in I	Number of colpodas reached a maximum after two days, about 50 animals counted in a 4 mm field using binocular dissecting microscope. Slight decline after four days.
					44	many	<i>Colpoda cucullus</i>	

* Identifications made by Dr. Gerald Thorne, nematologist, U. S. Department of Agriculture.

SERIES II

Number	Date	Medium	Amount of medium	Weight of hay	First observation of animals (in hours after starting culture)	Number seen at this time	Name	Remarks
I	Sept. 2	Hay infusion (.44 g timothy hay in glass distilled water—only clear fluid used)	100 cc	1.5 g	18½	1	<i>Colpoda cucullus</i>	Later history as in number III, Series I.
II	" "	Hay infusion. Same composition as above	9 cc	.13 g	23½	2	" "	Later history as in number V, Series I.
III	" "	" "	"	.07 g	23½	1	" "	"

SERIES III

I	Aug. 23	Tashmoo spring water	100 cc	.9 g	22½	1	<i>Colpoda cucullus</i>	By Aug. 25 culture was flourishing and continued with numerous colpodas until Aug. 27, when numbers decreased strikingly until almost no colpodas were present.
II	Sept. 2	" "	"	1.5 g	22	2	<i>Colpoda cucullus</i>	Maximum numbers reached Sept. 5 and declined rapidly thereafter.
III	" "	" "	9 cc	.13 g	23½	1	Nematode as above <i>Colpoda cucullus</i>	Later history as in number II of this series.
IV	" "	" "	"	.07 g	44	many	" "	"

SERIES IV

Number	Date	Medium	Amount of medium	Weight of hay	First observation of animals (in hours after starting culture)	Number seen at this time	Name	Remarks
I	Sept. 2	Hay infusion in Tashmoo spring water. (Same concentration as in Series II)	100 cc	1.5 g	20½	1	<i>Colpoda cucullus</i> Nematode as in Series I	Reached maximum concentration on Sept. 4; numbers declined after Sept. 5.
II	"	"	9 cc	.13 g	64	1	<i>Colpoda cucullus</i>	Numbers small at all times.
III	"	"	"	.07 g	23½	3	"	Numbers slightly greater than in No. II of this series, declining after Sept. 8.
	"	"	"	"	23½	1	"	"

todes from all but those of Series II. It should be mentioned that in addition to the organisms listed in the various series small flagellates which have not been identified occurred generally. It is interesting to note that at this period certain of the organisms, i.e., *Oxytricha*, *Spathidium* and *Physarum*, which were recovered in 1929, did not appear in any of the series. It is possible that these organisms may still be alive in the encysted condition. This seems highly improbable, however, since representative samples of the hay were used in every large culture. The distribution of the colpoda cysts generally throughout the hay is proved by the fact that from the very small samples used in the Syracuse watch-glass cultures colpodas were recovered in all cases except one (Culture IV, Series I). In this culture it is significant that no leaves of hay were present. It is believed that the round hay stalks used here did not afford an adequate surface on which the colpoda protection cysts could form.

The experiments were planned to test the relative merit of different kinds of media in inducing excystment and multiplication in the original medium after excystment. The records show that while organisms were recovered in motile condition in each type of medium, multiplication continued longer and a higher concentration of colpodas was reached in glass distilled water than in the media made from spring water. It is noteworthy, however, that the nematodes of the various genera identified multiplied only in the spring-water cultures. This multiplication went on even after the decline of the colpodas in these cultures.

In the rapidly multiplying colpoda cultures a great discrepancy in size was noted as in the previous paper, Dawson and Mitchell (1929). Small individuals measuring from 25 to 31 μ in length were isolated in sterile culture medium, and upon multiplication gave rise to individuals up to 50 μ in length. On the other hand, large individuals measuring from 55 to 60 μ in length were isolated from the original cultures and gave rise to

progeny of various lengths from 25 to 60 μ . Both large and small colpodas are identical in external morphological details. It is therefore certain that we are here concerned with but a single species of *Colpoda*. This discrepancy in size commonly occurs in rapidly dividing protozoan cultures. It was noted that the division cysts varied greatly in size while the cultures were flourishing, the usual number of organisms coming from each cyst being either two or four. In one case, however, a division cyst was observed from which eight colpodas of the smallest size (25 μ long) emerged.

This account constitutes a record of the greatest longevity of the protection cysts of *Colpoda cucullus* hitherto reported.

SHORTER ARTICLES AND DISCUSSION

THE INFLUENCE OF MANGANESE IODIDE AND ETHYL BUTYRATE ON RATS FURNISHED A VITAMIN A-FREE DIET

PREVIOUS studies from this laboratory having indicated that ferrous iodide exerts beneficial effects when furnished in minute quantities to rats on a Vitamin A deficient diet (Chidester, Eaton and Thompson, 1928), we decided to test manganese iodide also.

Thirty-seven young rats, about 30 days of age and averaging 35 gm in weight, were placed on a diet deficient in Vitamin A and low in Vitamin D (Sherman No. 380) on October 14, 1929, and when greatly depleted to the point of xerophthalmia and weight loss, were, on January 25, separated into lots with split litters and corresponding weights and isolated in individual round cages. With the exception of the controls, they were then furnished with 1/100 mgm of irradiated ergosterol daily from January 25 to February 18, when Lot 1 received 5 drops of MnI_2 and Lot 2 received 3 drops of MnI_2 in a solution such that the first lot received .0005 grain of I_2 and the second lot received .0003 grain of I_2 , as in an experiment with FeI_2 elsewhere recorded.

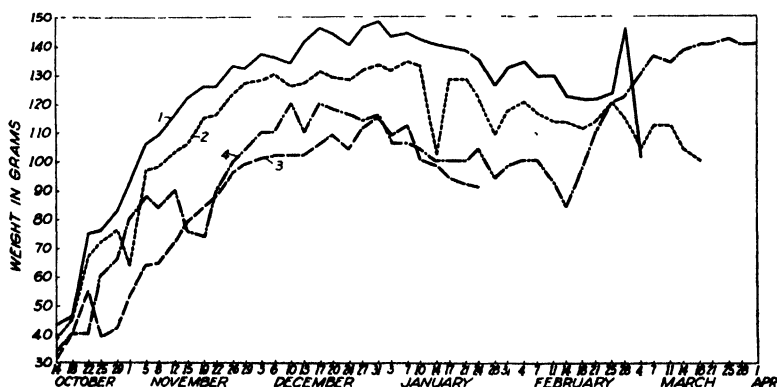


FIG 1 MANGANESE IODIDE AND ETHYL BUTYRATE, WITH ERGOSTEROL
Lots and Treatment

1. Sherman No. 380, ergosterol, January 25; 5 drops MnI_2 , February 18; 5 drops ethyl butyrate, February 22.
2. Sherman No. 380, ergosterol, 3 drops MnI_2 , 5 drops ethyl butyrate.
3. Sherman No. 380, Control.
4. Sherman No. 380, Cod Liver Oil, control.

RESULTS

Lot 1 continued to utilize stored foods and to increase in weight until December 31, then rapidly fell in weight and showed signs of deficiency in A and D until they were furnished ergosterol on January 25. From that date until February 6 the effect of the added Vitamin D was quite evident, but it rapidly wore away until on February 17 the average weight had gone down to 121 gm, the weight shown on November 14 before stored foods had been depleted. After the addition of MnI_2 on February 17, marked stimulation of growth occurred, the curve rising noticeably. Again, the average increased rapidly with the addition of 5 drops of ethyl butyrate on February 22. The death of all but three of the lot occurred by March 4, however. It would seem that manganese iodide is not so satisfactory a catalyzer as ferrous iodide.

Lot 2 became depleted rather rapidly from about January 10 to January 25, the date when they began receiving 1/100 mgm of irradiated ergosterol. The stimulating effect of this Vitamin D addition lasted until February 5, then the curve began to fall until a low point was reached on February 16. On February 18 the administration of 3 drops of MnI_2 (.0003 grain of iodine) daily raised the average 9 gm in 4 days. The addition of 5 drops of ethyl butyrate on February 22 failed to improve the condition of the lot, and on March 4 only 2 survived in a much depleted condition, although exhibiting no signs of xerophthalmia.

Lot 3, controls on Sherman No. 380 diet, were depleted in about 9 weeks.

Lot 4, a control lot, received ergosterol on January 25, and showed some slight effects, but began to grow rapidly when the ergosterol was replaced by one drop daily of cod-liver oil beginning February 14.

CONCLUSION

We feel that in this experiment we have possibly demonstrated a slight benefit from ethyl butyrate, added to a diet containing MnI_2 , but that we must seek other fats. Theoretically, we should be able to utilize highly unsaturated fatty acids which would balance the iodine if it should at any time exceed its usefulness as a catalyzer in vitamin-deficient rats.

Thirty-one young rats, about 30 days of age, averaging about 35 gm in weight, were selected for an experiment in which

minute quantities of MnI_2 were added to irradiate ergosterol and furnished animals that had been depleted for more than 8 weeks on a diet (Sherman No. 380) deficient in Vitamin A and low in Vitamin D.

The iodine content of the solution was such as had been proved beneficial in combination with iron, and elsewhere described, and was such that 5 drops of MnI_2 contained .0005 grain of iodine, and 3 drops contained .0003 grain of iodine. The solutions were delivered from pipettes graduated so that each drop represented 1/15 of a cc.

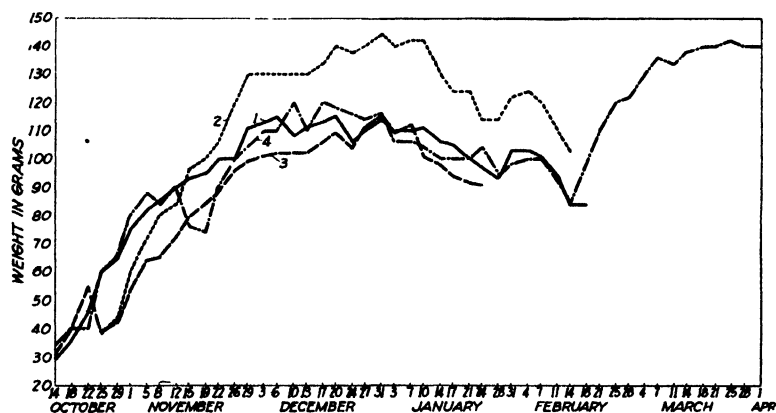


FIG. II MANGANESE IODIDE, WITH ERGOSTEROL

Lots and Treatment

1. Sherman No. 380; ergosterol, January 25; 5 drops MnI_2 , February 18.
2. Sherman No. 380; ergosterol, 3 drops MnI_2 .
3. Sherman No. 380; Control.
4. Sherman No. 380; ergosterol, January 25, replaced by Cod-liver Oil, February 14.

RESULTS

Both Lots 1 and 2 showed only slight stimulation of growth after the addition of ergosterol, and apparently could not tolerate the added MnI_2 . It is possible of course that we have underestimated the value of the ethyl butyrate added in Experiment 1 (Graph 1). Our general reaction, however, is that we must seek fats or fatty acids that will be less readily volatilized and that, although they are Vitamin A free, will aid in restoring our fat-iodine balance.

GENERAL CONCLUSION

These experiments, together with other preliminary studies performed with a small number of animals, would seem to indi-

cate that MnI_2 , given in the presence of irradiated ergosterol, does not prolong the lives of rats on an A-deficient diet more than six weeks and adds little to their growth, even when small quantities of ethyl butyrate are added.

In comparison with these results we have elsewhere (Chidester, Eaton and Thompson, 1930) discussed experiments in which ferrous iodide, with the same daily dosage of iodine as we have used in the present experiment, not only cured and protected rats from xerophthalmia, but induced growth and preserved the animals for more than fourteen weeks after profound depletion.

Studies of McCarrison (1927) have shown that MnO_2 in doses of .880 mgm per day caused retardation of growth in rats on a normal diet, the signs becoming evident at the end of 32 days. But $MnCl_2$ in daily doses of 0.0327 mgm for 53 days caused accelerated growth, particularly in the male rats.

Aso (1902) has shown that manganese sulphate in solution (0.002 per cent.) stimulates growth in radishes, peas and wheat plants, but that this effect is counteracted by the addition of iron to the solution.

Bishop (1928) showed that, without manganese, iron assimilation was normal in plants, but that growth ceased after 5 to 7 weeks. Calcium counteracted the toxicity of high manganese concentrations. It may be conjectured that in our experiments irradiated ergosterol made available a greater supply of calcium which reduced the toxic effects of the manganese. In McCarrison's studies it is also conceivable that the $MnCl_2$ brought in sufficient chlorine to act as an oxidant on the ferrous salts contained in the organs of his experimental rats.

While ethyl butyrate may prove beneficial in some deficiencies, it is probable that other fats or fatty acids will prove more palatable and perhaps more suitable, as slight increases in iodine dosage prove quite injurious to depleted animals. Again, we must consider the possible beneficial rôle of unsaturated fatty acids (Burr and Burr, 1930) presented with iodine (Chidester, 1930) under such profound nutritional deficiencies as we have experimentally produced.

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THE *CREPIS SETOSA* CHROMOSOMES PRESENT IN
CREPIS ARTIFICIALIS; A CORRECTION OF TISCH-
LER'S STATEMENT CONCERNING AUTOSYN-
DESIS AMONG *SETOSA* CHROMOSOMES

In his paper upon the correlation of cytology and taxonomy in plants Tischler (1929) refers to the origin of the chromosomes of *Crepis artificialis*, a description of which was published by the present writers. Tischler has obviously misunderstood the original description and consequently credited *Crepis artificialis* with a chromosome origin not found by the authors, who wish to take this opportunity of calling attention to the correct derivation of the chromosomes of this new species which was detailed in their paper (Collins, Hollingshead and Avery, 1929).

In the following quotation from Tischler's paper it is stated that the four chromosomes (haploid number) from *Crepis setosa* conjugated to form two pairs during meiosis in the species hybrid, *Crepis biennis* ($n = 20$) $\times$ *C. setosa* ($n = 4$).

Ganz eindeutig scheint mir aber für eine Bindung nicht homologer Chromosomen der Fall zu sprechen, den Collins, Hollingshead u. Avery (1929) beschreiben. Es handelt sich um den sogenannten *Crepis artificialis*. Er ist hervorgegangen aus der Kreuzung *Crepis biennis* (20) $\times$ *Cr. setosa* (4). Nicht nur die 20 Chromosomen der ersten Art schlossen sich autosyndetisch zu Paaren zusammen, sondern auch die 4 der zweiten.¹ Wir erhalten so 12 Gemini. Und es ist doch die Zerlegung der vier chromosomen in zwei einander "homologe" Paare mehr also unwahrscheinlich (page 47-8).

The following excerpts from our paper are given here to show that the statement regarding autosyndesis of the four *Crepis setosa* chromosomes was not warranted.

¹ Italics inserted by present authors.

In *setosa* the four chromosomes are easily distinguishable, figure 1a. One long chromosome has a subterminal constriction to which a large satellite is attached by a thin thread. A second, about equally long, has the subterminal constriction but lacks the satellite. The third is similar to the second but is shorter, while the fourth is very small with a subterminal constriction.

The two *setosa* chromosomes present in the *artificialis* complex are quite readily recognized by their morphological characters and each is present twice. These are the first and fourth types mentioned above . . . (p. 309).

In the F_1 resulting from crossing *artificialis* with *setosa*, plants containing 10 chromosomes from *biennis* and 6 from *setosa* were secured. The latter included the two pairs present in *artificialis* plus one member of each of the two pairs not represented in *artificialis* . . . (p. 316).

The *setosa* chromosomes in *artificialis* can be identified as the long chromosome pair with a large satellite and the smallest pair of *setosa* . . . (p. 319).

It was also pointed out in the paper referred to by Tischler and likewise in a previous publication (Collins and Mann, 1923) that in interspecific crosses involving *C. biennis* the haploid *biennis* chromosomes (20) conjugated to form 10 pairs but that the chromosomes of the other species remained as univalents and were distributed at random during meiosis.

It is hoped by this note to correct the statement that autosyn-desis took place in the haploid sets of both species which contributed to the origin of *Crepis artificialis*.

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LILLIAN HOLLINGSHEAD

PRISCILLA AVERY

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GENETIC NON-DISJUNCTIONAL FORMS IN DROSOPHILA¹

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DURING the past thirteen years the writer has been interested in a stock producing forms which differ from the normal in a variety of ways, but chief among which is the enlarged pattern of the ommatidia of the eye. The abnormal flies vary within themselves. Some have a coarse eye pattern and a slightly enlarged size throughout as the chief characteristic by which they may be separated from the normal. Another group shows changes in the sex organs and secondary sexual characters, another group has the wings cut and the wing veins irregular, the fly giving a wholly debilitated appearance. A further type appears as a small fly with slender short bristles. The appearance of these types suggests the individuals which Bridges has described as triploids, sex-intergrades, supersexes and haplo IV. They differ from his material, however, in their much more frequent appearance within this stock, where all types of these individuals may appear as often as once in thirty-five times. They further differ from his material in that the abnormal forms are due in last analysis to the action of a single gene. The purpose of this paper is to describe the anatomical structure of these different forms and to compare them with the normal.

¹ From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.

THE NORMAL

Fig. 1 shows the normal male and female *Drosophila* used as parents for the production of the atypical offspring.

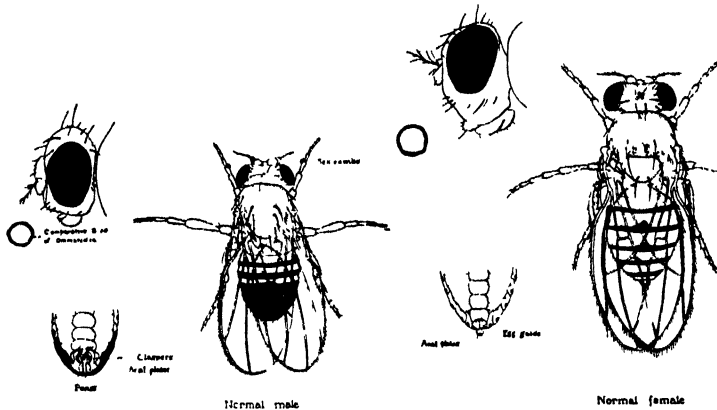


FIG. 1. The normal male and female *Drosophila melanogaster*.

The most important points of difference between the sexes visible in Fig. 1 are: the male is distinctly smaller than the female; the male has a black comb-like structure (sex-comb) on one of the joints or tarsi of the front legs, whereas these structures are lacking in the female. The abdomen of the male presents a less banded appearance than that of the female due to the difference in the distribution of pigment, although both are segmented. The external genitalia of the male are composed of plates of a shape very different from those of the female. These are known as the clasper plates and the anal plates. Those of the female are called the egg guide and anal plates. The relative size of the facets composing the compound eye is shown in the camera lucida outline of one facet.

The internal anatomy is equally distinct. The male had the following organs: two yellowish coiled tubes, the testes; two glandular structures, the paragonia, opening into the vas deferens, a duct leading from the testes and paragonia to the ejaculatory sac; the sperm vesicle,

ejaculatory sac; the ejaculatory duct leading from the sac to the penis; and the penis. The female organs are: the ovaries; ducts leading from each ovary and joining into a common duct, the oviducts; the uterus; two mushroom-shaped dark-colored organs, spermathecae, with ducts opening into the dorsal wall of the anterior end of the uterus and containing sperm after copulation; another receptacle for sperm known as the ventral receptacle; the parovaria; and vaginal portion of the uterus. The male and female flies which are the parents of the abnormal chromosome type appear to be entirely normal in respect to all these organs.

THE VARIANT TYPES

Within such a group of normal individuals coming from normal parents, there occasionally appears a fly which is a variant from type in one or many particulars. The changes may affect any or all of the organs mentioned. The affected flies are generally sterile. The sex instincts are altered, the duration of life materially shortened. The first general group are individuals which have within themselves both male and female organs. These sex-intergrades may be more or less

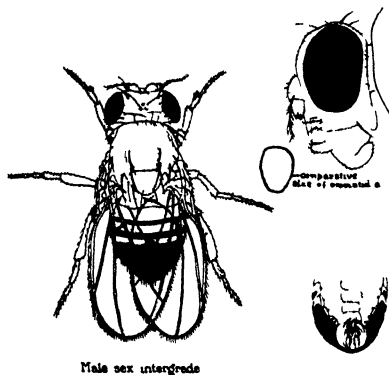


FIG. 2. Male sex-intergrade. This fly is seen to resemble closely the male externally. It differs chiefly in the pattern made by the facets of the compound eye which are large and therefore make the grid appear bigger. The outline of the facet size should be compared with the normal male as both are drawn to the same scale.

arbitrarily divided into three general types. Figs. 2, 3 and 4 show certain type-forms selected to show the extent of the changes in the external organs. These forms may be arranged arbitrarily from those most like the male to those most like the female. Fig. 2 shows the male type in appearance, the only striking change being the en-

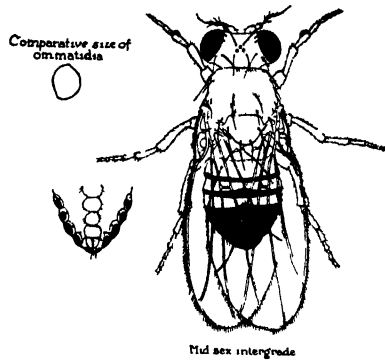


FIG. 3. Mid sex-intergrade. The noticeable features in the external appearance of individuals of this type are body appearance (sex-combs, size and pigmentation of abdominal plates) like the males while the genitalia are female. Eyes show the enlarged grid.

larged grid of the eyes formed by the increased size of the facets. The relative size of these facets is shown by the camera lucida outline of one of average size. It will be noted that the facets are much larger than those of the normal fly. Fig. 3 shows the intermediate type: the external appearance of the body being generally male, the external genitalia female, and the eyes with the enlarged grid. Fig. 4 shows the other extreme:—size of body and pigmentation of abdominal segments are of the female type. The sex-combs are generally present. Eyes show the enlarged grid. The genitalia are female type.

The types of changes which are indicated are also frequently accompanied by profound changes in behavior, both the male and female type often losing their sex instincts. These flies are uniformly sterile whether they resemble males or females in appearance. While

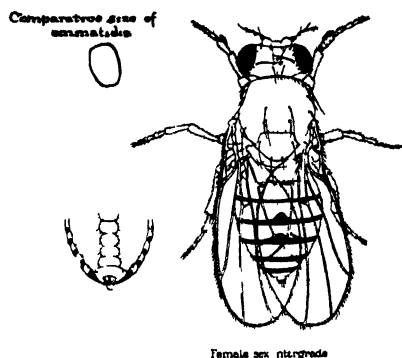


FIG 4 Female sex-intergrade. The type of body is female with certain alterations toward the male: sex-combs and sometimes male genitalia. The eyes have the enlarged grid.

the abnormal animals have been described as type specimens, it should be understood that all intergrades between normal males and females are to be found. They are sex-intergrades in the same sense as those described by Bridges.

Besides these sex-intergrades there is another abnormal type. These flies are generally larger than the ordinary females. They are entirely normal females in appearance save the grid on the eye which is enlarged. They are fertile but are capable of reproducing markedly different progeny than those of type males and females.

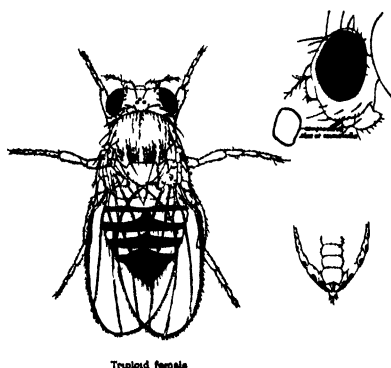
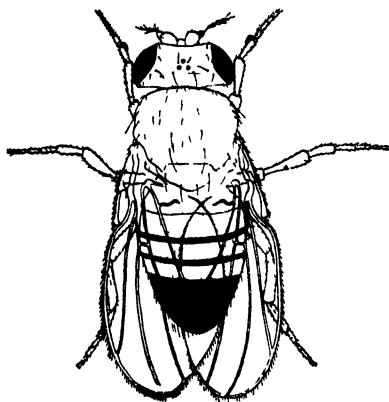


FIG. 5. Female type of fly having enlarged grid in eye pattern. Fly breeds as female reproducing normal males and females and a great excess of abnormal types shown in Figs. 2, 3, 4 and 5.

These progeny are of the following kinds; normal males and females, sex-intergrades of the three types described and females like themselves. These females are triploids.

Another type of fly with very fine eye grid and bristles is less frequently found among the progeny of these normal-appearing parents within this line of flies. These flies are physically weak and show a short duration of life. They may be of either sex. Both sexes are frequently sterile and when fertile are of low productivity. Fig. 6 shows a male of this type.



Slender bristles, male

FIG. 6. Male showing slender bristles and fine eye grid.

The last type of fly found less frequently than the preceding types has a forlorn appearance, wings cut, wing veins thickened and irregular, eyes bulging with a somewhat enlarged grid, body shrunken and female throughout. Duration of life is short. These flies are sterile.

In the sex-intergrades, the internal changes are more extensive than the external changes. The male-appearing sex-intergrades tend to have organs of more nearly the male type, the mid sex-intergrades the most pronounced abnormalities, and the female sex-intergrades tending to be more nearly like the females. Thus parents 134 had a male type of sex-intergrade among their

offspring. This male sex-intergrade showed a more or less typical male appearance—sex-combs, anal plates and clasper plates. The wings were cut out, a fairly frequent change in the body of such animals. The paragonia, vas deferens, ejaculatory sac, penis and testes were present. The left testis was very small and intensely yellow. The egg guide, spermathecae, ovaries, parovaria, uterus or ventral receptacle of the female were not present.

Fig. 7 shows a drawing of the sex organs of the male sex-intergrade type. One testis is fairly normal, the other much reduced in size and of abnormal shape resembling more nearly the testis as found in the pupa. Only one paragonium of reduced size was present. Vas deferens, ejaculatory sac and penis somewhat reduced in size. Clasper plates present. Flies of this type are sterile.

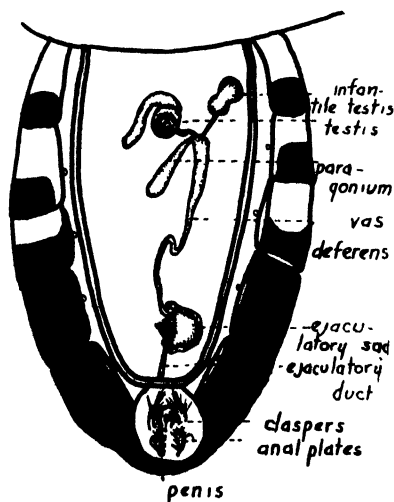


FIG. 7. Sex organs of male sex-intergrade.

In the mid sex-intergrade class, which is after all only the extreme of either class, was a fly from Female 132 which showed a general male appearance so far as the distribution of pigment on the abdominal segments was concerned. Sex-combs were present. The anal plates, egg guide, two spermathecae, ovaries (left one very

small, largely trachea), uterus and ventral receptacle of the female were present while the parovaria were absent. Of the male internal organs only a much deformed, small, yellow, right testis was present. In another such case from Female 539, the general appearance of the segments and body was female. The fly showed the following male organs: sex-combs, anal plates; one juvenile, yellow testis and one long, wrinkled, thin testis; vas deferens; and ejaculatory sac. The female organs present were egg guide, two spermathecae, parovaria, uterus and ventral receptacle. The male organs lacking were clasper plates, paragonia and penis. The female organs lacking were the ovaries.

Fig. 8 shows certain of the changes found in flies of the mid sex-intergrade type. What appeared to be a testis of the infantile type was present. No ducts or other male organs were present save the pigmentation of the abdominal plates, which was male in type. Anal plates were much deformed, clasper plates present. Two spermathecae and a ventral receptacle were present although much reduced in size. Uterus and female genitalia were present.

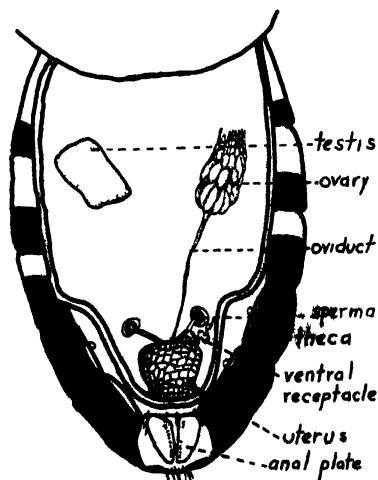


FIG. 8. Sex organs of a mid sex-intergrade. This figure should be compared with Fig. 6.

In the female sex-intergrade class there is somewhat less uniformity than in the male class. Thus one sex-intergrade from Female 102 had a general female appearance, so far as body and pigmentation of the segments were concerned. Sex-combs were present. Anal plates were much deformed, clasper plates present. Two small ovaries chiefly composed of tracheal tissue were present. Other normal organs of both sexes were absent. Another case from Female 133 had quite a normal female appearance, no sex-combs, wings cut, female anal plates, egg guide, two spermathecae, two ovaries of somewhat reduced size, parovaria, uterus and ventral receptacle. No male organs were present. In every case the eyes showed the enlarged grid.

Fig. 9 shows the generative organs as found in a female sex-intergrade. Only one ovary of much reduced size was present. The oviduct was small. Two spermathecae of small size present. The parovaria and ventral receptacle were absent. Uterus and external female genitalia present. The abdominal plates were of the female type.

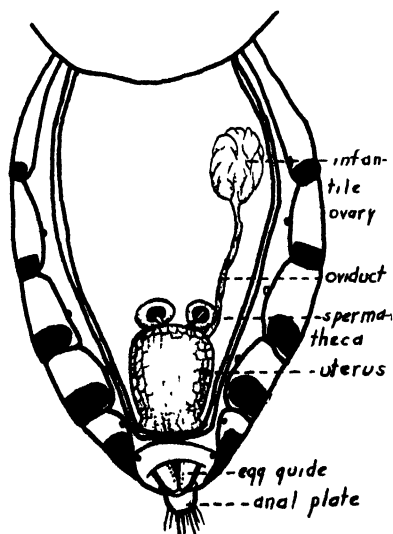


FIG. 9. Sex organs of a female sex-intergrade. This figure may be compared with Figs. 6 and 7.

TABLE I
ABNORMALITIES IN STRUCTURE OF CASE SPECIMENS

Number	Sex-combs	General appearance	Wings	Anal plates	Egg guide	Clasper plates	Spermathecae	Ovaries	Parovaria	Uterus	Ventral receptacle	Testes	Paragonia	Vas deferens	Ejaculatory sac	Penis
6278	+	♂ ♀	+	♂ ♀	-	+	-	-	-	-	-	left + right small	-	+	+	+
6278	+	♂ ♀	+	♂ ♀	-	+	-	-	-	-	-	small	+	+	+	+
6279	+	♂	+	♂	-	+	-	-	-	-	-	+	+	+	+	+
6296	+	♂	+	♂	-	+	-	-	-	-	-	+	1	+	+	+
6296	+	♂	+	abnor- mal	-	abnor- mal	-	-	-	-	-	+	small	+	+	+
6299	1 small	♂ +	+	♂ ♀	-	+	+	very small	-	+	-	-	+	-	-	-
6297	+	♂	+	♂	-	small twisted	-	-	-	-	-	1 very small	+	-	-	-
6296	+	♂	+	♂ ♀	-	-	-	-	-	-	-	small	+	+	+	+
6310	+	♂ ♀	+	♂ ♀	-	+	-	-	-	-	-	+	+	+	+	+
6324	+	♂	+	♂	-	+	-	-	-	-	-	+	+	+	+	+
134	+	♂	cut	♂	-	+	-	-	-	-	-	yellow small	+	+	+	+
117	+	♂	+	♂	-	+	-	-	-	-	-	yellow	+	+	+	+
112	+	♂	cut	♂	-	+	-	-	-	-	-	yellow small	+	+	+	+
114	+	♂	+	♂	-	+	-	-	-	-	-	yellow	+	+	+	+
171	+	♂	+	♂	-	+	-	-	-	-	-	+	+	+	+	+

TABLE I—(Continued)

Organ examined																
Number	Sex-combs	General appearance	Wings	Anal plates	Egg guide	(Clasper plates	Spermathecae	Ovaries	Parovaria	Uterus	Ventral receptacle	Testes	Paragonia	Vas deferens	Ejaculatory sac	Penis
115	+	♂	+	♀	-	+	-	-	-	-	-	small yellow	+	+	+	+
133	+	♂	+	♂	-	+	-	-	-	-	-	yellow	+	+	+	+
133	+	Mid	+	-	-	-	-	-	-	-	-	small yellow	+	+	+	+
133	+	♂	+	♀	-	+	-	-	-	-	-	yellow	+	+	+	+
133	+	Mid	+	-	+	-	-	small left ovary	-	-	-	small yellow right testis	-	-	+	+
132	+	Mid	+	♂	+	-	+	small left	-	+	+	small yellow right testis	-	-	-	-
132	+	♂	+	♂	-	+	+	+	+	+	+	+	+	+	+	+
133	-	♂	cut	♂	+	-	+	+	+	+	+	-	-	-	-	-
111	+	♂	+	♂	+	-	+	-	+	+	+	-	-	-	-	-
102	+	♂	+	♂	+	mixed	-	+	-	-	-	2 infantile	-	-	+	-
102	+	Mid	+	♂	+	-	2 small trachea tissue 1 small	-	-	-	-	1 small yellow	-	-	+	-
205	+	♂	cut	♂	+	-	+	-	-	+	+	2 small yellow	-	-	-	-
539	+	♀	+	♀	+	-	+	-	+	+	+	2 small yellow	-	+	+	-
924	+	♂	+	♂	-	+	-	-	-	-	-	+	1	+	+	+

A table, showing in somewhat diagrammatic form the changes found in a number of cases, is given. Histological examination of many of this type of flies frequently shows testicular and ovarian tissue present in the same organ.

The abnormal type shown in Fig. 5 has the female organs throughout. The only apparent difference between these organs and those of the normal female is that they give the impression of being slightly larger.

Flies of the type of Fig. 6 are either male or female throughout, no blending of the characters taking place. The sex organs are in general reduced in size, the flies frequently being infertile.

The last type of fly is female throughout and sterile. The ovaries and oviducts seem shrunken in appearance. Sections of the ovaries are atypical.

In their broader aspects the specimens described present a rather definite pathological picture, involving changes in the cell size, reproductive organs, etc. The group brought together as sex-intergrades are quite uniformly sterile. The type shown in Fig. 5 is fertile but shows its abnormal constitution in the fact that while breeding as a female progeny are produced which are of the normal and abnormal types.

A measure of the effect of these alterations from the normal may be had by studying the sum total effect as indicated by the time the individual's life mechanism is able to continue functioning. Experiments to determine the duration of life of each group have been performed and while of much interest in themselves need only be summarized here. The normal females live slightly longer than any other class. Individuals like those of Fig. 5 live a short time less than the normal females. The difference is not significant. The normal males live a somewhat less time. Individuals of the type of Fig. 2 have but half the duration of life of the previously mentioned classes and flies of the type of Fig. 4 live the shortest time of all, only one third the duration of life

of the other classes. These differences are all markedly significant and are of such extreme proportions as 3 to 1.

THE CHROMOSOME STRUCTURE

In the type male and female of *Drosophila melanogaster* it will be recalled that there are four pairs of chromosomes. The male has an X and Y, two pairs of long V-shaped chromosomes, the II and III, and a pair of small round chromosomes, the IV pair when seen in the metaphase plate. The female has a pair of rod-shaped X-chromosomes, two pairs of V-shaped chromosomes and a small round pair.

The normal-appearing males and females of the stock producing the sex-intergrades, triploids, slender bristled flies and super-females have been examined for their chromosome structure. Drawings of typical plates are shown in Fig. 10.

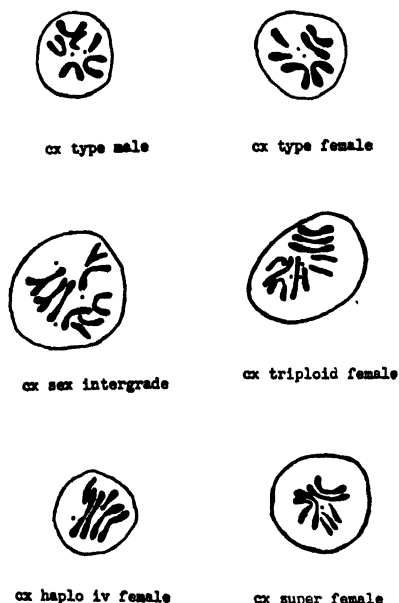


FIG. 10. The chromosomes of the male and female *Drosophila*, cx. stock, and sex-intergrades, triploid females, haplo IVs and super-females appearing as progeny from them.

The chromosome figures of the normal-appearing flies within this particular stock are seen in Fig. 10 to be essentially the same as those of the ordinary wild type stocks.

The female chromosomes differ from those of the male in that the female normally has two X-chromosomes and no Y-chromosome. Otherwise the female chromosomes II, III and IV are the same as those of the male. This is the most typical case of this particular stock. Occasionally the female cells show besides the two X-chromosomes a Y-chromosome like that of the male. This Y-chromosome appears to play no particular part in the further life of the fly; in fact, is simply a concurrent mechanical condition brought about by the atypical maturation processes of the egg formation. The normal males and females of this stock are therefore essentially like the males and females of wild types.

The cells of the sex-intergrades, figures 2, 3 and 4, show a triploid instead of a diploid complex, there being twelve chromosomes present instead of eight, two X-chromosomes, a Y-chromosome and three each of the second, third and fourth chromosomes. The fourth chromosomes are not always clear in all the figures, as they are small and difficult to differentiate. Fig. 10 shows the chromosome plate of such a form.

The changes which are found in flies of the type of Fig. 5 are similar to those in the other forms. Fig. 10 shows the chromosome plate of such a fly. The chromosomes are in triplicate throughout: three X's, three II's, three III's and three IV's, with occasionally a Y-chromosome. This arrangement of the chromosomes enables this fly to form normal eggs and diploid eggs. The dual nature of the eggs from these flies results in a repetition in the progeny of the types previously described, normal males and females, sex-intergrades and triploid females. This repetition of the different types in the progeny of such triploid females is proof that the abnormal chro-

mosome number is the underlying cause which is responsible for the abnormalities found.

The slender bristled flies are found to have a normal chromosome constitution, save in one particular; they have only one fourth chromosome. The low viability of such flies makes this difficult of proof in every case, but both the cytological figures found and the genetic tests possible all substantiate this conclusion. They apparently correspond to Bridges' haplo IV.

The debilitated females are triploid in the sex chromosomes but diploid otherwise. They are apparently identical to super-females.

THE GENE AS THE ETIOLOGICAL AGENT

Since the purpose of this paper is simply to present the anatomical aspects of the problem, it need only be said that the physical entity causing these extensive changes, when viewed grossly or microscopically, is a gene in the middle of the third chromosome.

DISCUSSION

The sex-intergrades, triploids, slender bristled weak males and super-females, as herein described, come directly from normal parents. A single pair of these normal flies may give 1 to 6 abnormal flies during the course of the culture, seldom more than that. These atypical flies give the first inkling of the abnormal conditions in the maturation processes of the eggs within the parents of this particular strain. All these atypical forms have an abnormal chromosome number within each of their cells. The extensive alterations from the well-known wild-type fly are due to the fact that these mothers gave them eggs having their peculiar chromosome numbers. The flies may be considered as primary non-disjunctional forms in which we are observing the origin of new types. The types described by Bridges, since they come from and as a mechanical consequence of this primary non-disjunction, are secondary non-dis-

junctional forms. It is of interest therefore to note the similarity of these individuals with the secondary non-disjunctional triploids, sex-intergrades, super-females and minute bristled flies as described by Bridges and by Dobzhansky and Bridges. So far as can be determined the types are identical, type for type, in their external appearance, internal structure and chromosome characteristics and even to the variability observed in the organ distribution of the intersexes.

It is, however, possible to obtain all types of non-disjunctional forms as primary individuals from this stock, whereas the secondary forms are less likely to be found all within one strain.

Within this strain the frequency of the formation of these primary non-disjunctional individuals varies from as low as 1 to 100 flies to as high as 1 to 30. This ratio is 100 to 300 times the rate found in ordinary stocks. Bridges has made the suggestion that when normal stocks have such an occasional primary non-disjunctional individual found in the progeny that such an individual comes from cells within the ovary which are markedly larger than the ordinary cells and carry more than the ordinary complement of chromosomes. This reasoning is based on the evidence that "in three separate preparations of ordinary 2 N females it was found that a portion of an ovary was constituted of markedly larger cells; and in two of the individuals some of the giant cells were in division, and the chromosomes could be counted as 4 N. Evidently there had been in some oogonial cell a division of the chromosomes that had not been followed by division of the nucleus and cytoplasm. The resulting tissue was tetraploid, and any reduced gamete would be 2 N. Such a 2 N gamete, fertilized by a normal sperm, would account for each of the twenty-five recurrences of triploid." In view of the increased frequency of the abnormal forms within the strain here described it would be expected that, if the hypothesis of Bridges were true, areas of large-sized cells and multiple chromosome num-

bers would be quite common within the ovaries of females from this strain. Such is found not to be the case. No enlarged cells have been observed within the ovaries of the normal parents. Neither have cells been observed with increased chromosome numbers. The evidence of the number of abnormal types per parental bottle furthermore point rather to the maturation division as the point where the non-disjunction takes place in forming these atypical chromosome flies at least for this stock.

The data herein presented support the contention that sex is determined as an interaction of the autosomal chromosomes and the sex chromosomes. The fact that within the intersex group there is such a wide variation in the degree with which the sex characters are developed and that this variation seems to be correlated with the type of gene complex found in the chromosomes further points to the conclusion that the gene elements within the chromosomes are the responsible factors and not the chromosome *per se*.

The cause of these changes is a third chromosome factor. To the extent that this factor modifies the end product of the reaction sex it could be looked upon as a sex factor. It is, however, probably better since it is one of the very few genes for which we have some information of its physical action to regard it rather as it really is—a factor whose presence or absence materially influences the maturation of the chromosomes within the female.

The cell size of the ommatidia of the eyes is shown beside the figure of the fly. Study of these brings out the fact that the cell size is correlated directly with the chromosome quantity contained within them. Dobzhansky's extensive study of this same question utilizing the cell size of the wing arrives at the same conclusion for the secondary non-disjunctional forms.

The recent republication of Boveri's hypothesis of the cause of cancer as due to abnormal chromosome numbers within the cancer cells and Metcalf's revival of this

hypothesis in his writings makes a consideration of these results from that view-point significant, since within the cells of the forms here described there is chromosome reduplication and chromosome unbalance. In outline Boveri's suggestion is based on the following reasoning: By fertilization of sea-urchin eggs with sperm in various ways abnormal chromatin forms could be produced. The cell size of these forms bore a correlation to the amount of chromatin contained in their nucleus. Mitoses were frequently abnormal. The number of chromosome combinations was very great, rivaling the number of types of tumors. Amitoses took place. The chromosome complex, incompatible in number, kind, or both, clearly caused these abnormal structural forms. As a parallel to this, cancer cells are frequently associated with enlarged cell size brought about by nuclear fusion. These cells may form irregular spindles and eventually pass into amitotic division. On this parallel Boveri based his suggestion. In a more general perspective, the case appears even stronger, for as Wilson points out the limitations of growth and cell-division, while they may be controlled by a variety of agents, such as available food and hormones, are in the last analysis governed by heredity. This is obvious from the fact that specific mean size of organisms is hereditary. Histological analysis has shown that it is the number of cells rather than their size which governs an organism's mass. Mendelian analysis has shown that this size is determined by the genes within the chromosomes. Or speaking more broadly, the limits of cell-division and, therefore, growth are determined by the chromosome constitution of the cells. The mechanism controlling the amount of cell-division is therefore present in the cell itself and should it be released might well give the uncontrolled cell multiplication so impressive in tumor formation.

The facts deduced from this investigation are of particular significance to this hypothesis of Boveri. In this

particular stock fertilized eggs are produced which in turn give rise to individuals which have 3 sex-chromosomes and 3 of each autosome group. While these individuals show a slightly larger cell size and body mass than the normal females of 2 sex-chromosomes and 2 of each autosome group, they are otherwise typically female in their organs, no noticeable abnormalities appearing. From this, the conclusion appears justified that the mere quantitative multiplication of the chromatin does not necessarily cause abnormal growths. The other type of abnormal eggs contains 3 sex-chromosomes, two of the X-type and one Y-chromosome, and 3 of each autosome. These individuals differ from both the normal male complex (one sex-chromosome of the X-type and one of the Y-type and 2 of each of the autosomes) and the normal female complex (2 sex-chromosomes of the X-type and 2 of each autosome group). Such adults are thus different in both the quantity and balance of the chromatin from the normal individuals. These individuals present abnormalities which are of wide range and clearly marked but they seem to result in a restraint in growth rather than a complete lack of its control. In fact, the abnormal organs formed and their peculiar association, *i.e.*, ovary and testis in the same animal or spermathecae associated with testes, are in general much reduced in size. In the germ cells, cell division and the normal maturation process appear hampered, but few cells going through the complete processes to egg or sperm formation, sterility due to this excessive cell restraint apparently always resulting. These results, therefore, tend to negative Boveri's suggestion if they are accepted as comparable material on which a test of his hypothesis could be made. These organisms do have a characteristic which might conceivably produce a difference in reaction from that Boveri postulates, namely, they presumably have all their cells with the given abnormal chromosome complex. The result might well be different if only certain of the cells were ab-

normal in their chromosomes and growing perhaps at the expense of the rest. Such cases, known in *Drosophila* as gynandromorph and mosaic flies, have areas which differ from the rest of the body areas in the number of their chromosomes without the production of abnormal growth. The results agree with Boveri's in showing that the chromosome complex markedly influences growth.

SUMMARY

This paper presents an analysis of the type parents and atypical forms found within a special stock of *Drosophila melanogaster*. The anatomical changes observed in the abnormal individuals of this stock consist very largely in over or under growth of the organs, especially those of the reproductive system, bristles and wings. They involve alterations in the testes, ovaries, paragonia, vas deferens, ejaculatory sac, oviduct, spermathecae, parovaria, ventral receptacle and uterus, as well as the external genitalia, sex-combs, clasper plates and anal plates. Fertility is reduced. The duration of life is shortened. Bristles are reduced and wings cut. These forms appear sporadically within a certain family or its near relatives. The ratio of the abnormal individuals to normal varies anywhere from 1 in 26 to 1 in 100. Profound alterations are found within the cells of these abnormal individuals. The chromosomes are increased in number, 12 chromosomes instead of 8 being ordinarily found in the cells. The anatomical changes within the abnormal flies are shown to be associated with the alterations of this chromosome number. The agent which brings about these changes is a third chromosome recessive gene.

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THE DECREASE OF CROSSING-OVER OBSERVED IN TRANSLOCATIONS, AND ITS PROBABLE EXPLANATION

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A TRANSLOCATION is a rearrangement of chromosomal material involving the transfer of a section of a chromosome from its normal location to a new locus. The first translocation observed in *Drosophila melanogaster* (Bridges, 1923), as well as most of the translocations described in this animal since then, are simple translocations. In simple translocations a chromosome is broken, and one of the resulting fragments is attached to a different chromosome, the recipient chromosome being intact. Recently also reciprocal translocations have been found in *Drosophila* (Sturtevant and Dobzhansky, 1930; Muller, 1930; unpublished data of Karpechenko and of Dobzhansky). In these cases two chromosomes were broken, and the resulting four fragments reunited so as to give two "new" chromosomes. The phenomenon of reciprocal translocation is evidently identical with that known as segmental interchange. Segmental interchange is described by Belling and Blakeslee in *Datura* (Belling, 1927; Blakeslee, 1927*a*, 1927*b*, 1929), by Burnham (1930) in maize, and postulated to occur in *Oenothera* and in certain other plants (Darlington, 1929; Cleland and Blakeslee, 1930; Blakeslee and Cleland, 1930; Håkansson, 1920; Meurman, 1929).

The transposed fragment of the donor chromosome is in most cases attached to the end of the recipient chromosome (Patterson and Muller, 1930; Dobzhansky, 1930*a*). Cases, however, are known in which a fragment of the donor chromosome becomes attached to the side of the recipient chromosome at a point between the spindle

fiber and the end of the recipient (Bridges, 1923; Hamlett, 1926; unpublished data of Sturtevant). In these cases a branched chromosome is formed (such a branched chromosome, however, has not been observed cytologically).

Of the two fragments formed by breakage of a chromosome, it is the one which does not include the locus of the spindle fiber attachment which becomes reattached to a different chromosome. The other fragment of the donor chromosome, which includes the locus of the spindle fiber, may remain free, or a fragment of some other chromosome may become attached to it. Thus, the formation of chromosomes having no spindle fiber attachment or more than one does not occur, or, more probably, such an event leads to formation of a chromosome which is unable to behave normally in mitosis, and is therefore eliminated.

In most of the translocations known, the fragment of the donor chromosome transposed onto the recipient chromosome includes one of the free ends of the donor. Recently at least two cases have been found in which sections taken from the middle of the second chromosome became attached to the Y-chromosome (Dobzhansky, 1930*b*, unpublished data of Rhoades and of Schultz), and the parts of the second chromosome lying to the right and to the left of the removed section reunited again. This kind of translocation is possibly related to the phenomenon of deletion (Painter and Muller, 1929; unpublished data of L. V. Morgan and of Dobzhansky). In the known deletions, sections of the X-chromosome not involving either end of the chromosome were lost, and the two ends reunited again, forming a new chromosome which is both genetically and cytologically shorter than the normal X-chromosome of *Drosophila melanogaster*.

Individuals carrying translocations in heterozygous form are in most cases phenotypically normal. This fact stands in accord with expectations, since flies carrying translocations have all the genes present in normal

flies. Some of the translocations, however, are different from normal flies in appearance. The "Pale" translocation of Bridges (*l.c.*) produces a dilution of the eosin eye-color. A reciprocal translocation recently found by the present author (unpublished) affects the eye-shape in a manner similar to the well-known gene Bar. This translocation represents an exchange of section between the second and X-chromosomes. The factor producing this change of eye-shape, called baroid, behaves as a recessive allelomorph of Bar, and its locus apparently coincides with the point at which the X-chromosome has been broken. Such a coincidence of the locus of mutation with the locus of the breakage of a chromosome is noteworthy. The fact that the majority of translocations, at least in *Drosophila*, are lethal when homozygous (Muller and Altenburg, 1930; Dobzhansky, 1930a), and that the lethal factors seem to be located usually at or very near the breakage point, suggests some connection between certain mutations and chromosomal breakages. However, some translocations in *Drosophila* are fully viable and normal in appearance in homozygous condition (Dobzhansky, 1929; also unpublished data).

It is obvious from the account given above that the phenomena classed as translocations are very diverse in nature. The study of translocations in *Drosophila*, however, shows that one property is common to most of them. This is the reduction of the frequency of crossing-over in the chromosome, or in the limb of a V-shaped chromosome, in which the breakage occurred, or in the chromosome or in the limb of chromosome to which a fragment of another chromosome becomes attached. The following review of the known translocations in *Drosophila melanogaster* shows how widely this effect on crossing-over is distributed.

The reduction of crossing-over is observed in flies that are heterozygous for translocations. Individuals heterozygous for a translocation carry a chromosome broken into two parts (the donor chromosome, A^+ and A^+ in Fig.

1), and a normal (*i.e.*, unbroken) homologue of this chromosome (A^1 , Fig. 1). One of the two parts of the

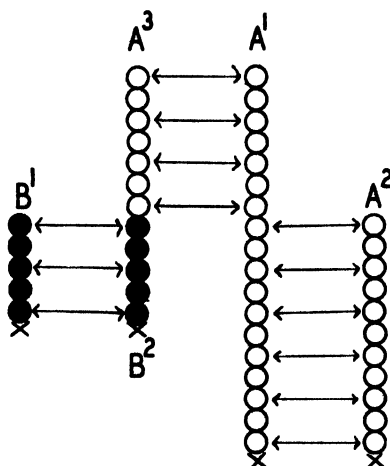


FIG. 1. A scheme of attraction between chromosomes involved in a translocation. The donor chromosome and its homologue are represented in white, the recipient chromosome and its homologue are in black. The locus of the spindle fiber is marked by $\times$. A^1 —the normal homologue of the donor chromosome. A^2 —the part of the donor chromosome preserving its own spindle fiber attachment. A^3 —the part of the donor chromosome attached to the recipient chromosome. B^1 —the normal homologue of the recipient chromosome. B^2 —the recipient chromosome. The arrows indicate the direction of the attraction forces between the homologous loci in different chromosomes.

donor chromosome, which does not include the locus of the spindle fiber attachment, becomes attached to the recipient chromosome (B^2 , Fig. 1). The other part of the donor chromosome (A^2), which includes the loci of the spindle fiber attachment, remains free. Crossing-over may occur between either section of the donor chromosome and its normal homologue. That is to say, crossing-over may occur between A^3 and the corresponding part of A^1 , as well as between A^2 and the corresponding part of A^1 . Sometimes double crossovers are observed in which the crossing-over occurred in both sections of the donor chromosome simultaneously. The appearance of such doubles proves that both parts of the

donor chromosome may conjugate with the normal chromosome simultaneously.

Five translocations involving the third and fourth chromosomes were studied in detail (Dobzhansky, 1930a). In all of them a section of the third chromosome was broken off and attached to the fourth chromosome. In three cases (*a*, *b*, *e*, see Table I) the third chromosome was broken between the spindle fiber (which is located at the locus of the gene peach, in the st-cu interval) and the left end (the ru end) of the chromosome. In these three cases a pronounced reduction of crossing-over was observed in the left limb of the chromosome (*i.e.*, between ru and peach), while in the right limb of the chromosome crossing-over remained normal, or was even slightly increased (see Table I). In two other cases (*c* and *d*, Table I) the third chromosome was broken to the right of the spindle fiber, and a reduction of crossing-over is observed in the right limb of the chromosome.

Four translocations involving a transfer of a section of the second chromosome to the fourth were found

TABLE I

DIFFERENCES BETWEEN THE CROSSING-OVER FREQUENCIES OBSERVED IN THE THIRD CHROMOSOME IN TRANSLOCATIONS, AND THE FREQUENCIES OBSERVED IN CONTROL

Interval		Control value	Translocations				
			<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>
Left limb	ru-h	23.7	-6.5	-2.5	+3.2	+5.4	-4.2
	h-D	13.7	-10.8	-7.8	+1.7	+1.1	-2.1
	D-th	1.1	-0.9	-0.9	+0.4	0.0	0.0
	th-st	0.8	-0.6	-0.5	+0.1	+0.1	-0.3
Spindle fiber	st-cu	8.0	-1.4	-3.4	-3.5	-2.1	-2.9
Right limb	cu-sr	15.4	+1.2	+1.7	-11.7	-2.8	+0.2
	sr-es	10.3	+1.4	-0.3	-2.8	-1.4	+1.3
	es-ca	31.0	0.0	0.0	-2.1	-11.8	0.0

(Dobzhansky, 1930*b*). In two of them (*a* and *d*, Table II) the breakage occurred in the right limb of the second chromosome, and a striking reduction of crossing-over is observed in this limb, while the crossing-over in the left limb is about normal. In two others (*b* and *c*, Table II) the breakage took place to the left of the spindle fiber (the spindle fiber in the second chromosome is attached at the locus of the gene *pr*), and a strong reduction of the frequency of crossing-over is observed in the left limb, while the right limb of the chromosome shows normal crossing-over. It is worth noticing that in all these cases the relatively strongest reduction of crossing-over is observed near the point of breakage of the chromosome (in the *pr* - *c* interval in *a*-translocation, in the *c* - *px* interval in *d*-translocation, see Table II), but in all cases some decrease of the frequency of crossing-over is observed in the whole limb in which the locus of breakage lies, and never in the opposite limb.

Four translocations involving the second and third chromosomes were studied (Sturtevant and Dobzhansky, 1930; Dobzhansky and Sturtevant, in press). In two of these translocations both the second and third chromosomes were broken at the spindle fiber, the left limb of the second and the left limb of the third chromosome united to form one "new" chromosome, and the two right limbs formed another "new" chromosome. Crossing-over was found to be decreased throughout the second and third chromosomes. However, if crossing-over in the second chromosome of these translocations is prevented by the crossover suppressors $C_{II\ L}$ and $C_{II\ R}$, the third chromosome shows the normal amount of crossing-over. Likewise, if crossing-over in the third chromosome in these translocations is suppressed by $C_{III\ L}$ and $C_{III\ R}$, crossing-over in the second chromosome remains normal.

In the third of these translocations the third chromosome is broken at the spindle fiber, but the second chromosome is broken to the left of its spindle fiber.

The right limb of the third chromosome is attached to the longer fragment of the second, and the shorter fragment of the second chromosome is attached to the left limb of the third. A strong reduction of crossing-over is observed in the left limb of the second chromosome, some reduction in the whole third chromosome, but the right limb of the second chromosome shows normal crossing-over. In a fourth translocation a section of the left limb of the second chromosome is attached to but not at the end of the left limb of the third. A striking reduction of

TABLE II

DIFFERENCES BETWEEN THE CROSSING-OVER FREQUENCIES OBSERVED IN THE SECOND CHROMOSOME IN TRANSLOCATIONS, AND THE FREQUENCIES OBSERVED IN THE CONTROL

Interval		Control value	Translocations			
			<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>
Left limb	{ al-dp	13.6	- 3.0	-13.3	-13.3	+ 0.1
	{ dp-b	31.0	- 0.2	-30.2	-21.4	+ 1.5
	{ b-pr	8.5	+ 0.1	- 8.0	- 2.4	+ 0.7
Spindle fiber {						
Right limb	{ pr-c	21.3	-19.5	- 1.9	+ 5.1	- 7.6
	{ c-px	23.8	-19.0	+ 2.8	+ 6.5	-13.8
	{ px-sp	7.1	- 3.3	+ 1.3	- 0.2	- 4.5

crossing-over is observed in the left limb of the second and in the left limb of the third chromosome, but crossing-over is normal in the right limbs of both chromosomes.

In the "Pale" translocation of Bridges (1923) a relatively small section of the right limb of the second chromosome is broken off, and attached to the right limb of the third chromosome, again not at the end of the chromosome. Hamlett (1926) found that crossing-over is reduced in the right limb of the third chromosome, but normal in the left limb of the same chromosome. No

data on the crossing-over in the second chromosome in this translocation are so far available.

Anderson (1929) has described a strain in which a high frequency of non-disjunction of the X-chromosomes is observed. According to unpublished data of Anderson, the high non-disjunction in this strain is due to the presence of a translocation involving the X- and third chromosomes. The locus of this translocation in the third chromosome is in the Delta-Hairless region, *i.e.*, in the right limb. Anderson found a reduction of crossing-over in the X-chromosome, and in the right limb of the third chromosome.

The present author has found a translocation involving the X- and second chromosomes. The X-chromosome is broken at the locus of Bar, and the second chromosome is broken at vestigial (*i.e.*, in the right limb), and the broken-off fragments of both chromosomes are exchanged. A reduction of crossing-over is observed in the right limb of the second chromosome, and at least in a part of the X-chromosome. The left limb of the second chromosome shows normal crossing-over.

Ten translocations involving the second and Y-chromosomes were found (Dobzhansky, 1930*b*, also unpublished data). In five of them a relatively small section of the right or the left limb of the second chromosome (including one of the ends of the chromosome) is broken off, and attached to the Y-chromosome. A reduction of crossing-over is found in every case in the limb of the chromosome in which the breakage took place, but nearly normal amount of crossing-over is observed in the opposite limb. In two translocations a section including the second-chromosome gene purple and some of the neighboring loci, but not including either end of the second chromosome, was transposed onto the Y-chromosome. In one of these translocations (studied by Mr. M. Rhoades) a reduction of crossing-over is found in the left limb of the second chromosome (in which the gene purple is located), but not in the right limb of the same

chromosome. In another case (studied by Dr. J. Schultz) some reduction of crossing-over is observed in the left limb of the second chromosome, but in the right limb crossing-over is nearly entirely suppressed. This nearly complete suppression is, probably, due to the presence of an inverted section, which arose simultaneously with the translocation. Finally, in three of the translocations involving the second and Y-chromosomes, nearly the whole limb of the second chromosome is broken off and reattached to the Y. In these three cases crossing-over in the second chromosome is normal.

According to Muller (1928*a*, 1928*b*) reduction of crossing-over is observed at least in some of the numerous cases of translocations studied by him. Oliver (1930) used the reduction of crossing-over as a method of distinguishing the gene mutations from chromosomal aberrations, including translocations.

The facts presented above may be summarized as follows: (1) If a chromosome is broken between its spindle fiber and its end, or if a fragment of another chromosome is attached to it, a reduction of the frequency of crossing-over in this chromosome is observed. (2) The relatively strongest reduction is observed in the intervals in or at which the breakage or the attachment took place. (3) In V-shaped chromosomes crossing-over in one limb need not be influenced by events taking place in the opposite limb. (4) If a V-shaped chromosome breaks into two equal parts (*i.e.*, if the breakage occurs near the spindle fiber), crossing-over in the resulting fragments may or may not be affected.

Several mechanisms may be suggested to explain these relations. It may be supposed, for instance, that an inverted section arises concomitantly with every translocation, and produces the observed change in the frequency of crossing-over. Such a situation may take place in some cases, but there is no known reason why inversions must always, or nearly always, arise together with translocations. Furthermore, reduction of cross-

ing-over is observed not only in case of breakage of chromosomes, but also when a section of a chromosome becomes attached to another, apparently intact, chromosome. Hence, an assumption would have to be made that a chromosome may become a recipient of a fragment of another chromosome only if it happens to carry an inversion. Finally, it is very doubtful whether an inverted section lying in a broken-off fragment of a chromosome can produce a decrease of the crossing-over frequency in the remaining part of the donor chromosome. Likewise, an inverted section lying between the spindle fiber and the locus of breakage can hardly be supposed to affect crossing-over in the broken-off fragment. The facts show, however, that crossing-over is reduced in the whole limb in which breakage took place. Thus, the inverted-sections hypothesis must be discarded.

The breakage of the chromosome, producing a change in the normal alignment of the chromosomal material, may be suggested to be the cause of the disturbance of crossing-over. This explanation is put aside by the results of the experiments of Rhoades (in press). Rhoades studied crossing-over in females having two normal second chromosomes, and a fragment (duplication) of second chromosome attached to the Y-chromosome. The presence of this fragment causes a reduction of crossing-over in the normal second chromosomes, which are unaffected by breakage. A similar result was obtained by the present author. Crossing-over was studied in females having two normal X-chromosomes, and, in addition to that, a fragment of X-chromosome (a deleted X-chromosome). Crossing-over in the normal X-chromosomes was found to be decreased as compared with a control experiment.

One may suppose that normal pairing of chromosomes, leading to normal crossing-over, may take place only if chromosomes first pair at the spindle fiber attachment. If so, the reduction of crossing-over in the broken-off section of a chromosome, which has a "new" spindle

fiber attachment provided by the recipient chromosome, becomes understandable. But this hypothesis does not explain the reduction of crossing-over which is observed between the locus of breakage and the spindle fiber in the donor chromosome. If the supposition is made that the ends of chromosomes determine the normal synapsis and crossing-over, a similar difficulty is encountered. For in this case reduction of crossing-over may be expected to occur in the donor chromosome, but not in the broken-off fragment. Moreover, neither of these hypotheses explains the reduction of crossing-over in the recipient chromosome, provided the latter is not broken.

A hypothesis, however, may be suggested which seems to fit the available data on reduction of crossing-over in translocations. An assumption must be made that the normal pairing of chromosomes, which is a prerequisite of normal crossing-over, is caused by the homologous loci (genes) in the chromosomes exhibiting at a certain moment of gametogenesis a specific attraction to each other.

Fig. 1 represents the donor chromosome A^2 , a section of which A^3 is broken off and attached to the recipient chromosome B^2 . An unbroken homologue of the donor chromosome, marked A^1 , and a homologue of the recipient chromosome, marked B^1 , are present in the same cell. The donor chromosome A^2 and its homologue A^1 are represented in Fig. 1 in white, the recipient chromosome and its homologue B^1 and B^2 are in black. The arrows show the attraction exhibited by the homologous loci.

Although the mutual attraction is ascribed to genes and not to chromosomes as wholes, chromosomes are known to be coherent bodies, and therefore must exhibit a certain degree of tenacity. Hence, the forces which pull a part of a chromosome in a given direction may be interfered with by other forces which may pull another part of the same chromosome in a different direction, and this is the situation in which certain chromosomes are in translocations. Chromosome A^2 is attracted

toward A^1 . The chromosome A^1 , however, is attracted to A^2 as well as to A^3 . If A^1 happens to lie between A^2 and A^3 , a conflict of the attraction forces is at hand. Hence, pairing of A^1 with either A^2 or with A^3 , or with both, may be retarded, or may never take place. The chromosome B^2A^3 will be in a similar situation if it happens to lie between A^1 and B^1 (see Fig. 1). Indeed, in this case the chromosome A^3B^2 is being pulled in two opposite directions at the same time. This scheme (Fig. 1), therefore, gives a graphic representation of a situation which may lead to reduction of the frequency of crossing-over between the spindle fiber and the locus of breakage in the donor chromosome (the locus of the spindle fiber is represented in Fig. 1 by a cross mark), as well as in the broken-off section (A^3), and in the recipient chromosome (B).

An objection might be made that the relative position of chromosomes represented in Fig. 1 is a rather special case, and that in other positions of chromosomes no conflict between different attraction forces arises. This is partly true, but if one takes into account the fact that at the moment of pairing the chromosomes are distributed in the nucleus in a three-dimensional space, this objection loses most of its force. For if chromosomes are represented lying in three dimensions, there are relatively few geometrical positions in which a conflict of the attraction forces is entirely eliminated. Moreover, in none of the translocations is crossing-over reduced to nil, which fact proves that in some cases chromosomes involved in translocations do pair successfully. It must be emphasized that presence of at least some crossing-over in all intervals in chromosomes involved in translocations constitutes a distinction between translocations and inversions. Indeed (Sturtevant, 1926, also unpublished data), no single crossing-over in the inverted sections of chromosomes can be recovered.

The reduction of crossing-over produced by inversions of sections of chromosomes can also be represented as

the result of a conflict of attraction forces. Sturtevant suggested in 1921 (Sturtevant, 1921) that if a section of a chromosome is inverted, and if a chromosome carrying an inversion is present in heterozygous condition (*i.e.*, along with a homologous chromosome having a "normal" sequence of genes), a reduction of crossing-over must result. In individuals which are homozygous for the inverted section the frequency of crossing-over must be normal. Inverted sections were actually found in *Drosophila* (Sturtevant, 1926, also a paper in press), and their genetic behavior was found to be in accord with expectations.

Fig. 2 represents a chromosome (marked A) carrying

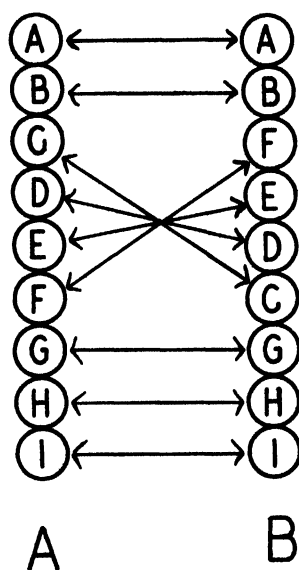


FIG. 2. A scheme of attraction between chromosomes differing from each other by an inverted section. CDEF—the inverted section. The arrows indicate the direction of the attraction forces between the homologous loci.

the genes ABCDEFGHI. If the CDEF section of this chromosome becomes inverted, a chromosome ABFEDCGHI arises (B, Fig. 2). The arrows show the direction of the attraction forces, which tend to

bring the gene A in contact with A, the gene B with B, C with C, etc. It follows from the inspection of Fig. 2 that in any geometrical position which chromosomes ABCDEFGHI and ABFEDCGHI may occupy in respect to each other some parts of one chromosome will be pulled away from the homologous parts of the other chromosome. It also follows from Fig. 2 that some reduction of crossing-over must be observed not only within the inverted section, but between the inverted section and the spindle fiber, and between the inverted section and the free end of the chromosome (*i.e.*, in the AB and in the GHI regions) as well. This is in agreement with experimental evidence secured by Sturtevant (*l.c.*).

The reduction of the frequency of crossing-over produced by breakages, by fragments of other chromosomes either free or attached, and by inverted sections is strongest in the neighborhood of the breakages, attachments and inverted sections, and becomes less pronounced in the parts of the chromosomes remote from the loci of these disturbances. This fact may be considered as a corollary of the attraction hypothesis. Indeed, the greatest conflict of the attraction forces must occur in the vicinity of the region of the chromosome which carries physical particles which are being pulled in opposite directions (see Figs. 1 and 2).

The effect of the inverted sections and translocations on crossing-over seems to be restricted to that limb of a V-shaped autosome of *Drosophila* in which the inversion or the translocation took place. The opposite limb of the same chromosome is unaffected. This fact may be partly accounted for by the weakening of the effect of breakages with distance (see above). The parts of the V-shaped autosomes adjacent to the locus of the spindle fiber (which is located in these chromosomes at the apices of the V's) are known to be much longer cytologically than suggested by the genetic maps (Dobzhansky, 1930*a*, 1930*b*). This discrepancy of the genetic and

the cytological maps is apparently due to the lower frequency of crossing-over per unit of the absolute distance in the vicinity of the spindle fiber as compared with the frequency of crossing-over in parts remote from the spindle fiber. Therefore, the effect of breakages on crossing-over must seem to become more rapidly weakened toward the spindle fiber, and less rapidly toward the free end of the chromosome. It may be questioned, however, whether the observed independence of the two limbs of the V-shaped autosomes can be completely accounted for by the factor just mentioned. Some facts seem to point in a different direction, suggesting that the homologous loci of the spindle fiber attachments possess a stronger attraction toward each other than other loci in the chromosomes. This point must be decided by further investigations. It must, however, be taken into account that the observed facts can not be explained by attraction of the loci of the spindle fibers alone (see above).

The value of the attraction hypothesis consists, primarily, in the fact that it can be tested experimentally. One such test is provided by the study of the interaction between the translocations and the inversions. Let us suppose that the chromosome B^1 (Fig. 1) carries an inverted section. In this case the attraction between the chromosomes B^1 and B^2 is weakened. Hence, the conjugation of the chromosomes A^1 , A^2 and A^3 may proceed with relatively little interference of forces pulling B^2 toward B^1 , and the frequency of crossing-over in A must rise. As shown above, this is found to be the case in translocations involving chromosomes II and III (Sturtevant and Dobzhansky).

A similar test is arranged in the following way. Suppose, for example, the chromosome A^1 (Fig. 1) carried an inverted section located within the part of the chromosome A^1 which is homologous to A^2 . In such an event the attraction between A^1 and A^3 would be weakened. Therefore, the pairing of the chromosome A^2 with A^1

would be no longer interfered with, and the frequency of crossing-over in A^2 must rise. An experiment conforming to the scheme just outlined was arranged, and gave the expected result.

A situation may arise when the attraction of the fragment A^2 to its homologue A^1 is so strong that it may overpower the attraction of the recipient chromosome B^2 to its homologue B^1 . Such a situation may be produced if the length of A^2 (Fig. 1) is much greater than that of B^2 , or if no chromosome completely homologous to B^2 is present in the cell. Translocations involving the second and Y-chromosomes seem to furnish an example of such a situation. It has been found (see above) that if a very long section of the second chromosome is attached to the Y-chromosome, the frequency of crossing-over in the second chromosome is normal or nearly so. If, however, a smaller section of the second chromosome is attached to the Y, the frequency of crossing-over in the second chromosome is decreased.

The supposition that the homologous loci in the chromosome attract each other at a certain moment of gametogenesis is by no means a new one. It is a well-known fact that the homologous chromosomes may lie far apart from each other during the premeiotic and the leptotene stages of meiosis. The chromosomes, however, shift toward each other, and during the pachytene stage appear as closely associated threads. It is known furthermore that the chromomeres which lie side by side in the pachytene stage are always of the same size and shape, while chromomeres following each other in a linear series may be of different size. These observations suggest so much the existence of attraction between homologous chromomeres that they were actually described in such terms by many cytologists. The works of Belling (1928), Darlington (1929), Gelei (1922), Metz (1925), Metz and Nonidez (1921), Reuter (1930) and Robertson (1930) may be referred to here. Most of the cytologists, however, use the expression "attraction" in

a merely descriptive sense; this is obvious from the fact that the expressions "attraction" and "affinity" are used indiscriminately by some authors.

The experimental evidence adduced in the present paper seems to furnish, however, a genetical counterpart to the cytological observations leading to the assumption of the existence of an attraction between the homologous loci in the chromosomes. It may be supposed that a physical force attracting homologous loci to each other at meiosis actually exists. The phenomena of translocation and inversion furnish a method of attack on the problems here involved. What is the nature of the force which attracts the homologous genes to each other is completely unknown at present. In most objects (except in those which manifest the so-called somatic pairing of chromosomes) this force is active only during the meiotic stages, but not at other times. Any speculations concerning the nature of these forces seem to be unprofitable at present.

The present work is based on the study of various translocations in *Drosophila*, which were carried on in years 1928-1930 at the California Institute of Technology. The author takes the opportunity to acknowledge his obligations to Dr. T. H. Morgan and to Dr. A. H. Sturtevant for their valuable suggestions and criticisms. Best thanks are due to Dr. Sturtevant in closest contact with whom the work was done.

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SOME ATTEMPTS TO OBTAIN HAPLOIDS FROM *OENOTHERA LAMARCKIANA*¹

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Oenothera Lamarckiana is believed to be a heterozygous species, the product of the union of gametes of different genetical constitutions, *gaudens* and *velans*, reproducing itself because in the main these are the only gametes formed which live and which give viable zygotes. If eggs of *Lamarckiana* could be induced to develop parthenogenetically and if the resultant haploids should be in some degree self-fertile it might be possible to obtain homozygous diploids which would help to solve the problem of the origin of *Lamarckiana*. With this in mind the writer in 1929 started some experiments which were concluded in 1930. The results, although negative, seem worthy of this report.

The chances were greatly against the success of the experiments for the reason that the gametes, *gaudens* and *velans*, carry lethals which will not allow the development of the zygotes *gaudens* · *gaudens* and *velans* · *velans*, these zygotes being represented by the more than 50 per cent. of sterile seeds found in *Lamarckiana*. It is to be expected that such lethals also would prevent the parthenogenetic development of *Lamarckiana* eggs although when brought together in the combination *gaudens* · *velans* their lethal powers are rendered ineffective. Nevertheless, there is the possibility that such lethals may at times be less harmful and it is even possible that lethals may become eliminated by gene mutations. In the event of such behavior the development of a haploid might be possible. For these reasons we may still hope that some one will be more fortunate and through experimental

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treatment or through crosses obtain from *Lamarckiana* fertile haploids.

Since Haberlandt reports that he has induced early stages of parthenogenetic embryos in *Lamarckiana* by pricking and pinching ovaries, this procedure was practiced over a period of four weeks on plants of *Lamarckiana* from de Vries's line and on plants of a line derived from wild *Lamarckiana* growing at St. Anne's, England. After cutting the styles of mature flowers before pollination, ovaries on the main stem were in some cases pricked with a needle and in other cases slashed with a razor blade one or more times. The treated plants were not allowed to set any fruit on side branches, thus giving the main stem the benefit of all food produced. There were 102 ovaries thus treated on the *Lamarckiana* of de Vries's line and 98 ovaries on plants of the line from St. Anne's. All of these ovaries withered and finally fell off from the mother plants.

The other line of experimentation involved the pollination of *Lamarckiana* from a number of forms, some of them wide crosses, in the hope that these pollinations might stimulate the parthenogenetic development of some eggs either in company with the production of hybrid embryos or in connection with parthenocarpy. The pollinations were made from the following species and I am greatly indebted to Professor Bartlett, who assembled the material at the Botanical Garden of the University of Michigan and identified it.

Hartmannia speciosa (Nuttall) Small. Reading, England.

Megapterium missouriense (Simms) Spach. Vaughan's seed store.

Raimannia odorata (*Oenothera odorata* Jacq.). Hort. Bot. Conimbrigensis, Portugal.

Raimannia Sellowii (Link and Otto) Dresden, Germany.

Raimannia Drummondii (Hooker) Rose var. *nana* Hort. Reading, England.

Oenothera argillicola MacKensie. White Sulphur Springs, West Virginia.

Godetia amoena (Lehm.) G. Don. Leiden, Holland.

Godetia purpurea G. Don. Lund, Sweden.

Lavauxia acaulis (Cav.) Spach. Leiden, Holland.

Lavauxia acaulis (Cav.) Spach forma *alba* Hort. Reading, England.

Kneiffia Youngii Hort., a variety of *Kneiffia fruticosa* (L.) Raimann. Vaughan's seed store.

Kneiffia Fraseri (Pursh) Spach. Vaughan's seed store.

All seed-like structures obtained from the pollinations, after soaking in water for a few hours, were subjected to that alternate pressure and exhaust which appears to force water through the seed coats and starts the immediate germination on wet filter paper in Petri dishes of all viable seeds. Plants of *Lamarckiana* and of *lata* appeared in some of the cultures. These were undoubtedly accidental selfings, for the work was done with buds just ready to open in order that the stigmas might be in favorable condition for the germination of foreign pollen and with the large number of pollinations made under this condition some selfing could hardly be avoided.

The results of the pollinations are given in the accompanying tables and fall into three groups: *First*, pollina-

TABLE I

POLLINATIONS OF LAMARCKIANA WHICH YIELDED NO SEEDS OR CAPSULES

Cross	Pollina- tions	Capsule set
<i>Lamarckiana</i> (de Vries) × <i>Godetia amoena</i>	18	0
<i>Lamarckiana</i> (St. Anne's) × <i>Godetia amoena</i>	16	0
<i>Lamarckiana</i> (de Vries) × <i>Godetia purpurea</i>	5	0
<i>Lamarckiana</i> (de Vries) × <i>Lavauxia acaulis</i>	14	0
<i>Lamarckiana</i> (St. Anne's) × <i>Lavauxia acaulis</i>	18	0
<i>Lamarckiana</i> (de Vries) × <i>Lavauxia acaulis</i> f. <i>alba</i>	16	0
<i>Lamarckiana</i> (de Vries) × <i>Kneiffia Youngii</i>	16	0
<i>Lamarckiana</i> (St. Anne's) × <i>Kneiffia Youngii</i>	10	0
<i>Lamarckiana</i> (de Vries) × <i>Kneiffia Fraseri</i>	16	0

tions from the species *Godetia amoena*, *G. purpurea*, *Lavauxia acaulis* and its form *alba*, *Kneiffia Youngii*, and *K. Fraseri* yielded no seed and set no capsules (Table I.). *Second*, pollinations from *Hartmannia speciosa* and *Megapterium missouriense* set seed-like structures which proved to be sterile, that is, contained no embryos as determined by examination following unsuccessful attempts to germinate them (Table II). *Third*, pollinations from *Raimannia odorata* (*Oenothera odorata* Jacq.), *R. Sellowii*, *R. Drummondi* var. *nana*, and *Oenothera argillicola* gave fertile seed in various proportions from which came obvious hybrids accompanied in some cases by occasional dwarfs that failed to mature; there were no forms suggestive of a haploid *Lamarckiana* (Table II).

During the past two years there have been four independent reports on haploid oenotheras. Gates (1929) announced the appearance of a haploid from the cross *O. rubricalyx* x *O. eriensis*. This plant at maturity had the appearance of a miniature *rubricalyx* but was almost completely pollen sterile, and the few seeds obtained by selfing failed to germinate. Gates and Goodwin (1930) give an interesting comparison of nuclear and cell measurements from various tissues of this haploid in comparison with those of the diploid *rubricalyx*.

Stomps (1930a) has obtained sterile *Hookeri* haploids from the crosses *Hookeri* x *longiflora* and *Hookeri* x *argillicola*. The cross *franciscana* x *longiflora* gave a *franciscana* haploid sufficiently fertile to make selfing possible. Seeds of the selfed haploid produced typical *franciscana*. The haploid pollinated by *franciscana* likewise gave *franciscana* but in addition several forms interpreted by Stomps as similar to the trisomic *scintillans* and *lata* and to the triploid *semigigas*. Stomps (1930b) also reports *argillicola* haploids from the cross *argillicola* x *biennis* in the high proportion of 16 haploids to 41 hybrids, but the chromosome count in these plants was not established. This seems the more remarkable

TABLE II
 POLLINATIONS OF LAMARCKIANA WHICH YIELDED SEEDS OR SEED-LIKE STRUCTURES

Cross	Pollina- tions	Capsules set	Seeds	Sterile seeds	Description of culture	Culture
Lamarckiana (de Vries) x						
<i>Hartmannia speciosa</i>	14	10 small	209	204	5 plants of Lamarckiana	30.101
Lamarckiana (St. Anne's) x						
<i>Hartmannia speciosa</i>	13	5 small	73	73	No germination	30.110
Lamarckiana (de Vries) x						
<i>Megapterium missouriense</i>	25	20 small	111	102	8 plants of Lamarckiana 1 plant of lata	30.102
Lamarckiana (de Vries) x						
<i>Raimannia odorata</i>	12	8 small	235	235	No germinations	30.103
Lamarckiana (St. Anne's) x						
<i>Raimannia odorata</i>	7	5 small	208	175	1 plant of Lamarckiana 1 hybrid 31 etiolated seedlings which died	30.111
Lamarckiana (de Vries) x						
<i>Raimannia Sellowii</i>	15	13 medium and small	539	515	24 tall, strong plants, evidently hybrids	30.104

TABLE II—(Continued)

Cross	Pollina- tions	Capsules set	Seeds	Sterile seeds	Description of culture	Culture
<i>Lamarckiana</i> (de Vries) × <i>Raimannia Sellowii</i>	15	14 medium and small	412	398	12 tall, strong plants, evidently hybrids 2 narrow leaved dwarfs	30.105
<i>Lamarckiana</i> (St. Anne's) × <i>Raimannia Sellowii</i>	14	14 small	758	410	348 seedlings but almost all were etiolated and died early 23 tall, strong plants, evidently hybrids	30.112
<i>Lamarckiana</i> (St. Anne's) × <i>Raimannia Sellowii</i>	16	12 medium and small	846	185	661 seedlings but almost all were etiolated and died early 2 plants of <i>Lamarckiana</i> 19 tall, strong plants, evidently hybrids	30.113
<i>Lamarckiana</i> (de Vries) × <i>Raimannia Drummondii</i> var. <i>nana</i>	17	17 medium	1775	1770	5 etiolated seedlings which died	30.106
<i>Lamarckiana</i> (de Vries) × <i>Raimannia Drummondii</i> var. <i>nana</i>	19	12 medium	806	780	26 etiolated seedlings which died	30.107

TABLE II—(Continued)

Cross	Pollina- tions	Capsules set	Seeds	Sterile seeds	Description of culture	Culture
<i>Lamarckiana</i> (St. Anne's) x <i>Raimannia Drummondii</i> var. <i>nana</i>	20	20 medium	1721	1280	441 etiolated seedlings many of which died early 239 weak, etiolated hybrids few of which matured	30.114
<i>Lamarckiana</i> (St. Anne's) x <i>Raimannia Drummondii</i> var. <i>nana</i>	14	14 medium	1977	1830	147 etiolated seedlings many of which died early 6 plants of <i>Lamarckiana</i> 62 weak, etiolated hybrids few of which matured	30.115
<i>Lamarckiana</i> (de Vries) x <i>Oenothera argillicola</i>	18	18 medium	2550	1310	1240 seedlings produced a culture of vigorous hybrids except for a few etiolated plants	30.108
<i>Lamarckiana</i> (de Vries) x <i>Oenothera argillicola</i>	16	14 medium	953	290	663 seedlings produced a culture of vigorous hybrids except for a few etiolated plants	30.109
<i>Lamarckiana</i> (St. Anne's) x <i>Oenothera argillicola</i>	15	15 medium	1305	249	1056 seedlings produced a culture of vigorous hybrids except for a few etiolated plants	30.116

since he did not obtain haploids from *argillicola* pollinated by *Hookeri*, *muricata*, *Lamarckiana* and *Lamarckiana-blandida*.

Emerson (1929) published, in 1930, a report on a haploid *franciscana* almost completely sterile from the cross *franciscana* x *franciscana-sulfurea*, and gives an account of anastomosing threads in the early stages of the spirem during meiosis and of twisted loops during the second contraction. It has been suggested by Cleland and others that similar conditions as found in diploid *Oenotheras* offer a cytological basis for crossovers, but Emerson points out that this suggestion does not find support in the haploid where these stages are also found, although homologous chromosomes are not expected to be present. It may, however, in fairness be noted that the possibilities of crossing over at such periods of meiosis in the diploid are not excluded by the findings in the haploid.

Davis and Kulkarni (1930) have described the genetics and cytology of a haploid that has appeared four times since 1923 in a selfed line of *Oenothera franciscana* with a frequency of about 1:1000. Three generations of selfed haploids have also been grown consisting chiefly of *franciscana* but with the haploid again appearing together with a number of other forms. The haploid was also noted from the crosses *franciscana* x *franciscana-sulfurea*, *franciscana* x *franciscana-sulfurea* dwarf, and (*franciscana-sulfurea* dwarf x *franciscana*) x *franciscana*. These crosses are between closely related plants and are not to be thought of as involving a stimulus from the pollen of distant types. The haploid back-crossed to *franciscana* gave *franciscana* as was to be expected. The chromosome count of the haploid was established in 1928 from a plant that appeared in 1927 in the line of *franciscana*, and an account of microsporogenesis was presented. Davis and Kulkarni (1930) also reported a sterile haploid plant that appeared in 1927 out of a line of *Oenothera*

Hookeri that included three generations and totaled 1,291 plants.

A line of *franciscana* was started in 1929 through seeds from the original haploid plant selfed in 1923. Such *franciscana* plants synthesized by the selfing of a haploid should be strictly homozygous and the behavior of such a line becomes a matter of interest. The plants themselves can not be distinguished from the wild *franciscana* of the original line which has been carried through fifteen generations. From this synthesized *franciscana* the first generation of 818 plants was grown in 1930 and proved to be uniform *franciscana* except for two haploids which flowered and one narrow-leaved dwarf rosette that died early. Thus the ratio of haploids in this line of synthesized *franciscana* starts out higher than that of the wild *franciscana*.

Other cases of parthenogenesis are known in *Oenothera* and are described by Stomps (1930*b*), but they concern the appearance of diploids from tetraploids and have not yet received the close cytological study which they deserve. Physiologically, such diploids would be expected to be more vigorous than haploids, and meiosis should not present so much irregularity in its procedure. They constitute a class quite apart from the haploids and seem likely to have peculiarities of their own.

It was suggested at the beginning of this paper that the chances of obtaining haploids from the heterozygous *Lamarckiana* may not be favorable because of the lethals carried by its gametes. *Oenothera franciscana* and *O. Hookeri* are in contrast homozygous species with all pairing chromosomes and with pollen almost wholly good and seeds more than 90 per cent. viable. Such species might be expected to give haploids more readily and they have been found independently by Stomps, Emerson, and Davis and Kulkarni. *Oenothera argillicola* from which Stomps reports numerous haploids following pollination by *biennis* is probably also homozygous since its pollen

in my examinations has been about 85 per cent. good and its seeds about 80 per cent. viable. *Oenothera rubricalyx*, which gave a haploid to Gates, has three pairs of chromosomes together with a circle of eight (Cleland), and its pollen is only 50 per cent. good and its seeds about 40 per cent. viable. These facts indicate that *rubricalyx* is heterozygous, a conclusion supported by its breeding behavior. If *rubricalyx* may give haploids it becomes a matter of interest to learn what may be the limitations to the production of haploids among the heterozygous species of *Oenothera*.

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A PRELIMINARY REPORT ON SOME CHROMOSOME ALTERATIONS BY X-RAYS IN *CREPIS*

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On the basis of extensive observations the following classification of the various types of chromosome alterations have been noted by the writer:

1. *Quantitations*: Processes leading only to change of the chromosome number without any alteration of the chromosomes themselves. Subdivisions of this class are:

- (a) Summations, i.e., addition or subtraction of entire haploid chromosome sets (polyploidy and haploidy);
- (b) Combinations, i.e., addition or subtraction of entire individual chromosomes (trisomy, polysomy, monosomy, etc.).

2. *Dislocations*: Rearrangement of chromosome material without any alteration in the total mass of the nucleus. Here should be classified various alterations known to occur spontaneously, such as attachment, duplication, translocation, fragmentation, etc.

3. *Transformations*: Alteration of the morphological characters of the chromosomes invariably connected with a change of the total mass of the nucleus, although often affecting different chromosomes independently.

Transformation is believed to have occurred in course of the evolution of the *Crepis* species, which in most cases markedly differ in the sizes and shapes of their individual chromosomes.

4. *Novations*: A presumed phenomenon of formation of the chromosomes *de novo*, which was called to account for some apparently otherwise inexplicable cases of appearance of new large "V-shaped" chromosome structures. As will be shown in the following, this last type should very probably be merged with dislocations.

While quantitations and dislocations were relatively often (in 1.0 and 0.1 per cent. of cases, respectively) found to occur spontaneously among individuals of the same species, the third type, viz., transformation, never was met with in a pure species. There seemed to be a lack of connection between the individual chromosome variation and the transformational process or processes which brought about the *specific* differences of the chromosomes.

In order to investigate the nature of these individual chromosome variations, an attempt was made to increase the frequency of their occurrence by means of X-ray treatment. In the following are reported the first results of these experiments.

Grateful acknowledgments are made here to R. A. Golonsko, M.D., and to M. A. Vassilevsky, M.D., who kindly made possible access to the necessary apparatus and helped in administering the X-rays.

As material for this investigation *Crepis tectorum* was chosen because it has only four pairs of chromosomes, each being morphologically clearly distinguishable from the others; also because it was known to throw many viable chromosome aberrations. Seeds (achenes) of a certain strain (No. 579), after having been soaked for twenty hours on wet filter-paper in a Petri dish, were exposed to various dosages of X-rays. The conditions of the experiment were as follows:

Distance from the target	20 cm
Kilovolts	108
Milliamperes	4.0
Aluminum filter	.5 mm

Exposures were made in the following way. All the seeds (after the control group was removed) were placed under the tube and exposed for forty seconds. Then, after having disconnected the current, a portion of seed was removed and the remaining seeds exposed again, this time for eighty seconds. Then the current was disconnected again and the second portion of irradiated seeds was removed, after which the remaining material was exposed for one hundred and twenty seconds. Thus the treatment was continued until the tenth portion. The exposures thus rapidly increased in the form of a progression, the tenth portion of material being irradiated totally for 2,200 seconds.

The peculiarity of the treatments administered in the above manner consisted in their discontinuity, for the

dosages, beginning with the second portion of seed, consisted of 2, 3, 4—10 separate exposures; alternating with short periods of rest during which the subsequent portions of treated material were removed.

After having been treated in the above manner the seeds were planted in pots containing soil. All ten lots germinated in a normal way and the seedlings did not differ from those which came from the untreated (control) seed of the same strain. Very soon after the germination, however, the treated individuals displayed marked influences of X-rays. These influences appeared as early as six days after germination and consisted in delayed or abnormal development of the first leaves. The proportion of such abnormalities was clearly connected with the dosages employed. While the first portions, treated with shorter exposures, showed but few abnormalities, the last portions contained only very few apparently normal individuals. The tenth portion,

TABLE I

DATA ON THE DOSAGES OF X-RAYS ADMINISTERED TO DIFFERENT PORTIONS OF SEED OF *Crepis tectorum* AND ON THE GERMINATION AND FIRST STAGE OF DEVELOPMENT OF THE SEEDLINGS

Portion number	Culture numbers	Exposure in seconds	Number of seeds	Number germinated	Died in early stages	Appearance of the young plants
0 (control)	30 × 1-0		10	9	...	
1	30 × 1-1	40	11	11		Occasional abnormalities
2	30 × 1-2	120	10	8		“ “
3	30 × 1-3	240	10	7		“ “
4	30 × 1-4	400	10	9		“ “
5	30 × 1-5	600	10	9		“ “
6	30 × 1-6	840	10	8		“ “
7	30 × 1-7	1,120	10	8		“ “
8	30 × 1-8	1,440	11	11	3	Many abnormal
9	30 × 1-9	1,800	10	10	3	The majority abnormal
10	30 × 1-10	2,200	36	26	13	All abnormal

treated altogether for 2,200 seconds, consisted only of abnormal individuals, a considerable part of which died prior to forming the first leaf. The above data are tabulated in Table I.

In the following will be given only a short account of the observations made on somatic chromosomes in the root tips taken from the plants belonging to the last three groups of this experiment. All other data on the treated plans as well as on reduction phenomena and on other experiments performed will be published elsewhere.

The cytological examination of root tips taken from the plants nearly three months old revealed an extraordinarily high proportion of altered individuals. The average percentage of these was as high as 60 for the three groups of plants, while the last group (exposed for 2,200 seconds) contained no normal individuals at all. It thus appeared that the X-ray treatment employed increased the "mutation" rate about 600 times on the average (the natural rate of spontaneous dislocations being *ca* 0.1 per cent., as was noted above).

All the chromosome alterations induced by the treatments represented the result of dislocations. No single case of quantitation was observed. The great majority of these alterations appeared in chimera seedlings, the most extreme individuals producing roots of as many as six karyologically different types. In two cases, however, identical chromosome alterations were found to be present in all the roots subjected to cytological investigation. This seems to furnish evidence in favor of the theory that the whole root develops from a single initial cell.

The normal complement of *C. tectorum* consists of four pairs of chromosomes which differ one from another as to size, place of the kinetic constriction (place of spindle fiber attachment) and presence or absence of satellites (see Fig. 1, *i*). Since the constrictions are connected with the kinetic functions of the chromosomes, they are

oriented with respect to the center of the equatorial plate so that the longer arms of the chromosomes are directed more or less toward the periphery. The shorter arm of the chromosome consequently is always turned toward the center, its end being thus the "proximal" end of the chromosome and the longer arm terminating at the "distal" end. The "A-chromosomes" consist of two arms, one of which is approximately twice as long as the other, the kinetic constriction being consequently located between the second and the third thirds of the chromosome length, counting from the distal end. The "B-chromosomes" are somewhat longer than the A's but possess subterminal constrictions, thus being composed of arms one of which exceeds the other in length about 6 to 7 times. The "C-chromosomes" are considerably shorter than the B's, but their shorter arms are slightly longer than in the B's, the constriction being located somewhat farther from the proximal end. The "D-chromosomes" have highly reduced shorter arms which appear as very small round "knobs," the constriction being located very near to the proximal end. The D's possess in addition satellites, very small spherical appendixes connected with the chromosome knobs by thin threads.

The nature of the chromosome alterations induced by the treatments is shown in Fig. 1, representing somatic chromosomes from the root tips of several mutated individuals. One may see from this figure that the alterations always involve not less than two chromosomes, since a portion of one chromosome is detached and transferred to another homologous or non-homologous chromosome. Proximal fragments of various sizes become autonomous diminished chromosomes, whereas the distal fragments invariably appear fused with other chromosomes. The only reason for such behavior is found in the fact that the distal fragments possess no kinetic constrictions and are eliminated unless they associate with some other chromosome supplied with a constriction. It

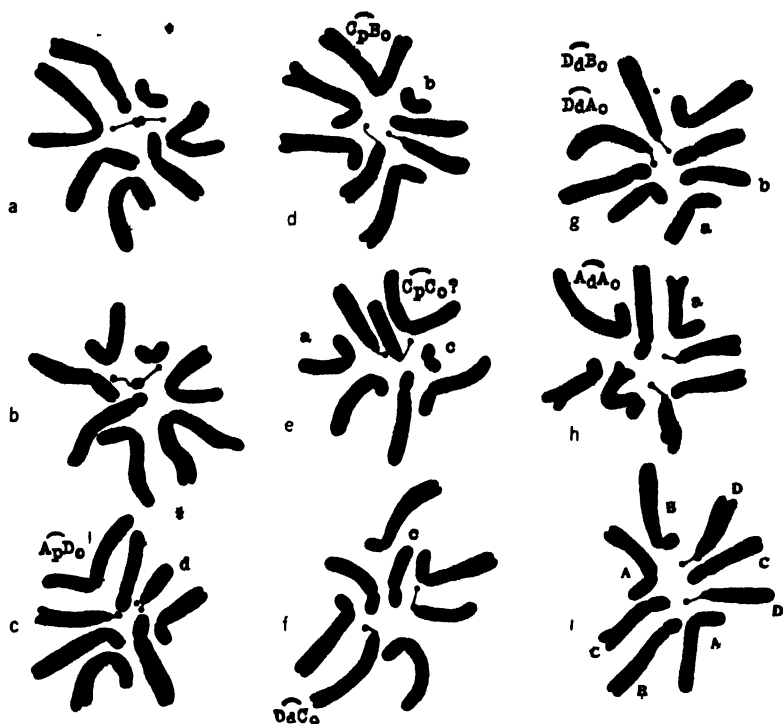


FIG. 1. Somatic chromosomes from the root tips of the plants derived from seeds exposed to X-rays for 1,440 to 2,200 secs.; a, b, very complex dislocations involving the majority of the chromosomes (the small bisatellited body being the product of fusion of the satellited fragments of the two D-chromosomes); c, $A_p D_o + d$; d, $C_p B_o + b$; e, $C_p C_o + a + c$; f, $D_d C_o + c$; g, $D_d A_o + D_d B_o + a + b$; h, $A_d A_o + a$; i, normal diploid complement of *C. tectorum*.

In all cases small letters indicate free proximal fragments containing a kinetic constriction; capital letters with the subscript o, distal fragments deprived of constrictions; horizontal brackets, fusion; subscripts p and d, the proximal and the distal ends of the chromosome, respectively, where fusion takes place. Capital letters without subscripts indicate the four types of the normal chromosomes. In addition to the dislocations indicated above, there are probably minor alterations present in some cases (in Fig. 1 d, for instance, the smaller arm of the free B-chromosome appears to be somewhat enlarged). See also text, p. 247. Magnification ca. 1,700 diameters.

should be noted, further, that normal unaffected chromosomes were never observed to fuse together. On the contrary, fusion was invariably connected with fragmentation. This seems to indicate that both processes (i.e.,

fragmentation and fusion) are due to some common cause, most probably related to some sort of interchange between non-homologous chromosomes.

In order to make possible a brief description of the alterations observed, the following system of designation was used. A detached proximal fragment containing the kinetic constriction was designated by a corresponding small letter. To designate the distal fragment of the same chromosome the same capital letter was used with the subscript *o*. Thus, for example, the proximal and distal fragments of a D-chromosome were designated by "d" and "D_o," respectively. To indicate fusion, a horizontal parenthesis was used. Finally, to indicate the point of fusion (in case it could be clearly determined) subscripts *p* and *d* were applied, signifying the proximal and distal ends, respectively. Thus, for instance, the formula $A_pD_o + d$ (Fig. 1 c) signifies that the distal part of the D-chromosome had been transferred and attached to the proximal end of an unaltered A-chromosome, while the proximal fragment of the same D-chromosome became an autonomous small chromosome.

The particular instances of alterations of individual chromosomes are explained in the legend for Fig. 1.

From cases represented in Fig. 1, as well as from many others which were observed, it might be concluded that there seemed to be no limitations as to size of the dislocated chromosome fragments. Nor did there seem to be any regularity as to the direction of the process. On the contrary, in the comparatively small amount of material examined (altogether 26 plants) nearly all possible combinations were found, so that it could be deduced that probably parts of any chromosome, of any size, can be transferred to any other chromosome and attached either to the proximal or to the distal end of the same.

The kinetic constriction, on the other hand, exhibited an outstanding stability. No cases where a constriction disappeared or was formed on a new place were found

in these experiments.¹ Nor were chromosomes with more than one constriction formed. It seems thus very probable that the main reason for the stability of chromosome number in a given group of related organisms (when polyploidy is excluded) is the above noted high stability of the kinetic constrictions, the number of which always corresponds to the number of chromosomes. It is not meant by this that the constriction can not be destroyed or changed but merely that the chromosomes lose their vitality or their ability to function normally when the kinetic constrictions are damaged or altered in some fundamental way. Loss of the normal constriction results in elimination of chromosomes so affected or of their fragments, unless they become associated with some other chromosome having a constriction.

It should be noted in this connection that dislocation of the chromosome material is not associated in any way with the formation of chromosome constrictions. As was shown in Fig. 1, in no case could any trace of constriction be seen at the place where a detached fragment was fused with the end of another chromosome. Experimental evidence thus seems to contradict the hypothesis that constrictions represent places of end-to-end fusion which might have taken place in the course of evolution. As for the question whether the dislocations represent only rearrangements of chromosome material or whether they can lead also to gain or loss, nothing definite can be said at the present time. Judging from what is known for other organisms, it seems rather probable, however, that cases of the latter sort should be not infrequent. At any rate, it is clear that quantitative variation in the total chromatin material would necessarily arise in the progeny of altered individuals due to recombinations of dislocated chromosomes. And, if viable, some of these descendants may obtain chromosome comple-

¹ The only case of *de novo* formation of the constriction in the distal fragment of the fragmented D-chromosome in *C. tectorum* was established by the writer in 1926.

ments transformed in the way which is characteristic of many related species.

Irrespective of their kind, the observed chromosome alterations did not seem to affect in any way the vitality of the cells or organs involved. Nor did even the most conspicuous dislocations (such as those represented in the Fig. 1 *a* and *b*) seem to influence seriously the growth or the size of the roots. It was thus evident that the various dislocations involving very different quantities and qualities of the chromatin material did not produce any "positional effect." On the contrary (at least in the presence of some unaltered chromosomes), all the roots, the cells of which were affected in different ways by chromosome dislocations, developed in an apparently normal manner.

As was shown above, the products of dislocations induced by X-ray treatment often assume a form of large "V-shaped" (two-armed) chromosomes usually composed of products of fragmentation fused with normal chromosomes. As was also stated, similar structures have been observed to occur spontaneously, in some cases even in association with obvious chromosome fragmentation. Due to some complications (loss of fragments, presence of extra chromosomes, etc.), the first observations on these spontaneous dislocations led the writer to an erroneous interpretation of the phenomenon in question. It was deduced, namely, that the occurrence of the new "V-shaped" chromosomes must be attributed to some peculiar process of *de novo* formation ("Novation"). At the present time when the experiment here reported produced essentially the same products through dislocations, there can be hardly any doubt that "Novation" represents nothing but dislocation involving large chromosome fragments.

It is of interest to note in this connection that the frequent formation of the V-shaped chromosomes through dislocation makes it possible that such chromosome

shapes occurring naturally, as they do in a great many species, may represent secondary structures of more recent origin. If such is the case, it would seem to contradict the hypothesis advanced by some writers that the chromosomes were primarily V-shaped. Such evidence, however, must be considered in connection with all other available evidence on the phylogeny of groups of species.

It is premature as yet to draw definite conclusions from the above data. It may be suggested, however, that dislocations should play a decisive rôle in species formation. Firstly, because they cause reorganization of the linkage relations which in most cases must inevitably result in a certain degree of cross sterility and even in practically complete physiological isolation due to altered affinity and disturbed conjugation of the dislocated chromosomes. Secondly, because dislocations may lead also, under certain circumstances, to chromosome transformation such as that postulated in the explanation of the evolution of chromosome structure. As a matter of fact, it is easy to see that subtraction or addition of varying amounts of chromatin material, differing in quality in different chromosomes and at different levels of individual chromosomes, opens up vast possibilities for genetic variation which would furnish material for the operation of natural selection and other evolutionary forces. Further investigations on the progeny derived from the treated individuals are in progress and new experiments are under way. Particular attention will be devoted to the obtaining of balanced (homozygous) types possessing pairs of identically dislocated chromosomes: to experiments bearing upon the problem of the time when induced chromosome alterations take place, and on the mode of their occurrence.

INHERITANCE AND LINKAGE RELATIONS OF CHOCOLATE PERICARP IN MAIZE¹

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SOURCE OF MATERIAL

AN open pollinated ear of chocolate maize was obtained from Mr. F. D. Richey, of the U. S. Department of Agriculture. The source of this ear was unknown, but the growth habit and plant color (weak purples and weak sun reds) of its progeny suggest a South American origin. Linkage tests have been made with all the known linkage groups using chocolate from this source.

During 1924 seed of chocolate maize was collected by Richey and Emerson at Sicuani, Peru. This chocolate was crossed with an early corn (New Brunswick flint) obtained from Mr. Richey, and has also been tested with all the known linkage groups. While the identity of the character from the two sources has not been completely established, the similarity in appearance and the failure of both stocks to show linkage with any of the nine known linkage groups indicate that the same gene is involved.

DESCRIPTION

Chocolate pericarp color is a simple dominant to colorless. The pericarp develops a dull grayish brown to chocolate pigmentation unlike any of the pigments commonly found in maize. A typical chocolate pericarp over white endosperm, compared with Ridgeway's "Color Standards and Nomenclature," approximates the color Drab 17¹¹¹¹. The cob also is colored. This is most clearly shown when the cob is broken. When

¹ Paper No. 176, Department of Plant Breeding, Cornell University, Ithaca, New York.

homozygous, the pigmentation is usually somewhat more intense than when heterozygous. The symbol *Ch* is suggested for the chocolate gene.

There is ordinarily no difficulty in classifying chocolate in mature ears except in the presence of self red, brown or cherry pericarp color, and even very immature ears show the color on drying. Purple and dilute purple plants occasionally show enough purple color in the pericarp to interfere. With homozygous purple aleurone identification of chocolate pericarp is difficult though

TABLE I
SUMMARY OF LINKAGE TESTS INVOLVING CHOCOLATE PERICARP COLOR

Genes	Phase*	XY	Xy	xY	xy	Total	Recombinations	Per cent. of recombinations
<i>Ch-C</i>	C	240	254	281	283	1058	535	50.6
<i>Ch-Sh</i>	C	17	5	27	10	59	32	54.2
<i>Ch-Wx</i>	C	31	6	34	9	80	40	50.0
<i>Ch-R</i>	C	163	185	174	202	724	359	49.6
"	R	203	172	209	177	761	380	49.9
<i>Ch-G</i>	C	72	45	55	42	214	100	46.7
<i>Ch-Su</i>	C	422	382	442	400	1646	824	50.1
<i>Ch-Tu</i>	R	28	30	20	24	102	52	51.0
<i>Ch-B</i>	C	837	858	867	833	3395	1725	50.8
"	R	175	175	170	172	692	347	50.1
<i>Ch-Lg</i>	C	146	151	143	151	591	294	49.7
"	R	42	53	52	52	199	94	47.2
<i>Ch-Pl</i>	C	159	169	162	152	642	331	51.6
"	R	314	329	316	332	1291	646	50.0
<i>Ch-Y</i>	R	279	288	319	313	1199	592	49.4
<i>Ch-Ts₁</i>	C	122	118	124	120	484	242	50.0
<i>Ch-F</i>	C	10	11	9	13	43	20	46.5
<i>Ch-Ea</i>	C	105	83	102	88	378	198	52.4
<i>Ch-A</i>	C	387	425	425	413	1650	850	51.5
"	R	38	57	49	50	194	88	45.4
<i>Ch-Ts₄</i>	C	59	80	70	68	277	150	54.2
<i>Ch-D₁</i>	C	50	25	45	13	133	70	52.6
<i>Ch-Cr</i>	C	50	25	43	15	133	68	51.1
<i>Ch-Pr</i>	C	191	182	200	207	780	382	49.0

* C = coupling and R = repulsion.

usually not impossible. In such cases cob color is helpful in the classification.

The development of chocolate pigmentation appears to be independent of the anthocyanin flavonol pigment system inclusive of the red and brown pigments of the pericarp. It develops uniformly on all plant color types. It also develops in the presence of other pericarp colors, though it may be obscured by them. Ears having the genes for red variegation and for chocolate show red stripes on a chocolate ground.

LINKAGE TESTS

A summary of linkage tests involving chocolate pericarp color is presented in Table I. These tests involve one or more genes from each of the known linkage groups. There is no indication of linkage with any of these nine groups. Some of these tests include two genes of the same group simultaneously. These tests are summarized in Table II. In addition F_2 data have been obtained from crosses of *Ch* with *gl*₁, *v*₅ and *v*₂. In no case is there any indication of linkage.

DISCUSSION

The linkage tests reported indicate that the chocolate gene does not belong to any of the nine known linkage groups. It may therefore represent the remaining tenth group. But since crossing-over occurs freely in both megasporogenesis and microsporogenesis, linkage tests alone can not with certainty exclude a gene from any linkage group. It always remains possible that the known linkage maps do not cover a sufficient portion of the chromosome and that, therefore, apparent independence may only mean that the gene in question lies at some distance from the known or tested portion of the map. This uncertainty is illustrated by the recent results of Brink and Senn (in press) and of Holmeyer (unpublished) in which *d*₁ and *cr* are united in one group with *a* *ts*₄ and *na* through the intermediate genes *Rg*

TABLE II
LINKAGE TESTS INVOLVING TWO KNOWN GENES IN THE SAME
CHROMOSOME

Order of genes	Crossover regions			
	0	1	2	1-2
<i>Ch</i>				
<i>sh wz</i>	34	6*	32	8*
<i>Ch</i>				
<i>sh wz</i>	34	32	8*	6*
<i>Ch</i>				
<i>su Tu</i>	37	15	32	20
<i>Ch</i>				
<i>su Tu</i>	37	32	20	15
<i>B</i>				
<i>lg Ch</i>	72	33	63	31
<i>B</i>				
<i>Ch lg</i>	72	63	31	33
<i>Pl Ch</i>				
<i>Y</i>	186	66	206	80
<i>Ch</i>				
<i>Y</i>	186	206	80	66
<i>Ch</i>				
<i>a ts₄</i>	62	65	72	78
<i>Ch</i>				
<i>a ts₄</i>	62	72	78	65
<i>Ch</i>				
<i>d₁ cr</i>	55	10	60	8
<i>Ch</i>				
<i>d₁ cr</i>	55	60	8	10

* Germination of shrunken seeds was poor.

(ragged) and *ba₁* (barren stalk), respectively, corroborated by McClintock by means of trisomic ratios involving *a* and *cr*.

The independence of the majority of the known groups has been established by means of aberrant endosperm (Emerson, 1924) or by trisomic inheritance (McClintock and Hill, 1931). Both of these methods are independent of map distance and should therefore be decisive. It is hoped that the tenth group represented by chocolate may be subjected to similar rigid tests in the near future.

RÉSUMÉ

Chocolate pericarp color in maize is a simple dominant to colorless. Tests with the nine known linkage groups give no indication of linkage. The gene *Ch* is therefore submitted as representing the tenth linkage group.

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ORIGIN OF MAMMALIAN FAUNAS AS ILLUSTRATED BY THAT OF FLORIDA

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THE study of evolution as a sequence of historic events, a fundamental aspect sometimes ignored by the biologist, is concerned with three things: origin, or change in structure; migration, or change in geographic distribution, and extinction. The underlying causes of these changes, the field of the evolutionary biologist, must be considered in the light of a knowledge of what the changes actually were, the field of the paleontologist. To consider what can happen, or how changes may occur, without reference to what actually has happened, is to divorce the science from reality.

In seeking to account for the origin of living animals there are numerous possible approaches. Many of these, including some of the most valuable, deal with isolated animals or single processes, or go to the opposite extreme of dealing with abstract principles without respect to actual causes. These may be checked and coordinated by the use of a method which has not, I believe, been fully expressed or appreciated. The data for this method would consist of an exact knowledge of successive animal societies, faunas, in a single region. The interpretation of these data would involve an elucidation of the differences and resemblances between these faunas. The ultimate aim would be the discovery of the causes for these relationships.

It is the present purpose to give an example of this approach toward the general problem of evolution. The facts are presented so far as they are known. The interpretation of these facts is certainly incomplete and probably erroneous in part, but the facts themselves are

concrete data on which theories may be based or against which they may be checked.

The example chosen consists of successive faunas, Pleistocene and Recent, of a limited area, the State of Florida. This example is taken because the faunas are fairly well known, much better than most faunas, and because the area is one naturally delimited and of especial intrinsic interest. Attention is directed to the land mammals, because these are by far the best known and because they do constitute a natural faunal group.

ORIGIN AND COMPOSITION OF THE PLEISTOCENE FAUNA

The best-known fossil fauna from Florida is that of the so-called Melbourne beds, and attention will be confined to species that are known to be derived from deposits of this age. From the circumstance that it contains most of the living species of Florida, it is probable that this fauna is of late Pleistocene age. Some authorities consider it as considerably older, but that is not important from the present point of view. It is a fauna of contemporaneous land mammals which preceded the recent fauna, by however long a period.

As now known to me, and after careful revision, this Melbourne fauna includes at least sixty-six species of land mammals; distributed ordinally as follows:

Marsupialia	1	Edentata	7
Insectivora	3	Perissodactyla	6
Rodentia	17	Artiodactyla	11
Carnivora	17	Proboscidea	4

This represents unusually complete knowledge for an extinct fauna, yet it doubtless does not include all the species then present here. It is probable that there were at least ten more species, a majority of which were probably small rodents and most of the rest probably carnivores.

It is reasonable to estimate that at least seventy-five species were present, of which at least twenty were rodents and almost as many carnivores. For the most

part, however, analysis may be based on what is known, with this suggestion as to the probable nature of the undiscovered part of the fauna.

The ultimate origin of this fauna is fairly well known. The species are all exclusively North American, and a majority of them were probably confined to the east or southeast. Ten species (edentates and three rodents), about 15 per cent. of the known fauna, belong to groups introduced from South America in the Pliocene or early Pleistocene. Nine species (among rodents, artiodactyls and proboscideans), about 14 per cent., belong to groups probably introduced from Eurasia not earlier than the late Pliocene, and perhaps some others belong here. The rest, about 70 per cent., appear to be old residents or truly autochthonous. This mingling of mammals of at least three very distinct geographic origins is an essential and striking factor in the Pleistocene fauna.

ORIGIN AND COMPOSITION OF THE RECENT FAUNA

In considering the recent fauna it is proposed to ignore subspecies and to include only land mammals probably inhabiting continental Florida in early historic times. The fragmentary nature of the materials and the lack of distributed geographic samples make determination of subspecies (in the sense of recent zoology) almost impossible to the paleontologist, and if they were considered the faunas would not be comparable.

On this basis, using some personal judgment as to the species recognized, there were thirty-three land mammals in the late prehistoric fauna of Florida, ordinarily distributed as follows:

Marsupialia	1	Carnivora	11
Insectivora	3	Artiodactyla	1
Rodentia	17		

Twenty-three of these species also occur in the Pleistocene. Their immediate origin is obvious. The ten spe-

which are not known from the Pleistocene are as follows:

- Sciurus niger* (Fox squirrel).
- Glaucomys volans* (Flying squirrel).
- Peromyscus* (four species) (White-footed mice).
- Pitymys parvulus* (Pine mouse).
- Mustela vison* (Mink).
- Canis floridanus* (Wolf).
- Felis coryi* (Cougar).

The rodents are all small animals, some indeed minute, and would be little likely to be preserved as fossils or, if preserved, to be found. They may well have been present in the Pleistocene. Their wide distribution and local differentiation in the state suggests that they are old residents. The apparent absence of the mink in the Pleistocene may also be accidental, as it is a small animal and one which was probably not common. The weasel is known from only two or three fossil specimens.

The absence of the two large modern predaceous carnivores in the Pleistocene may be more significant. If *Canis floridanus* is correctly distinguished from *Canis lycaon*, this fact in itself might stamp it as a fairly old inhabitant. Large canids are, however, rather common in the Pleistocene, and so far as determinable they appear to belong without exception to the extinct subgenus *Ænocyon*, the so-called dire wolves. Some of the indeterminate remains may represent the true wolves, or these may have been present but not discovered as yet. It seems clear that dire wolves were predominant among large Pleistocene canids and that true wolves were relatively few or may possibly, but improbably, not have entered Florida until Recent time.

Felis coryi, the other large predaceous carnivore of Recent Florida, is also unrecorded in the Pleistocene. It also is said to be a species peculiar to Florida, and may be an old inhabitant. Cougars do occur in the Pleistocene, close relatives of *F. coryi*, and it is possible that some of them have been incorrectly distinguished from that species. The question remains open.

About two thirds of the present fauna is known to have been derived directly from the Pleistocene fauna of the same region. Of the remaining third it is possible that one or two species are post-Pleistocene immigrants, but it is probable that all were derived from Pleistocene inhabitants.

In most or all of such cases as permit close comparison, the recent species are identical with their Melbourne age forerunners. Post-Melbourne evolution has on the whole been of subspecific or lesser value.

The ultimate origin of the Recent fauna is less complex than that of the Pleistocene fauna. No species belong to South American groups, and perhaps three, about 9 per cent., are of relatively late Eurasian origin. The rest are probably autochthonous in North America.

COMPARISON OF PLEISTOCENE AND RECENT FAUNAS

Successive faunas in the same region show differences due to three sorts of changes: (1) Immigration of new species; (2) evolutionary advance or differentiation of stocks already present; (3) extinction or migration of groups present in the earlier fauna.

It has been shown that immigration of new species accounts for little if any of the difference between the Melbourne and Recent faunas in Florida. Evolutionary differentiation has doubtless brought about some changes in subspecies but seems to have affected the species but little or not at all. The very great difference between these two successive faunas is thus almost or quite entirely due to extinction or emigration. The Recent fauna is simply a part, probably about two fifths, of the Pleistocene fauna. It has little or nothing that is new, except its poverty and limitation.

The problem of derivation of the Recent fauna thus becomes one of extinction. The recent fauna owes its character wholly to the fact that some Pleistocene species became extinct while others survived. This extinction had a differential character, affecting some groups more

than others, entirely removing three orders, for instance, while the rodents were comparatively little affected. Also within the orders it spared some species and removed others apparently equally fitted for survival. This differential effect of extinction in determining the character of a fauna is worthy of tabulation and study. Its general nature in this case is made clear in the following table.

Order	Pleistocene species not present in the Recent fauna	Percentage of total Pleistocene-Recent species of this order
Marsupialia	0	0
Insectivora	0	0
Rodentia	7	29
Carnivora	9	45
Edentata	7	100
Perissodactyla	6	100
Artiodactyla	10	91
Proboscidea	4	100

Certain of the species belong to genera now extinct everywhere, while others represent more or less local extinction of groups surviving elsewhere. The extent to which this extinction was local may be judged from the following table.

Order	Total known in Pleistocene	Genera extinct	Genera living but not in Florida	Known Pleistocene genera living in Florida
Marsupialia	1	0	0	1
Insectivora	3	0	0	3
Rodentia	16	2	4	10
Carnivora	14	3	2	9
Edentata	6	5	1	0
Perissodactyla	2	0	2	0
Artiodactyla	7	4	2	1
Proboscidea	3	3	0	0

Of the living genera not now present in Florida, seven (*Thomomys*, *Ondatra*, *Synaptomys*, *Erethizon*, *Vulpes*,

Canis [*Thos*], and *Bison*) have ranged north and west of Florida in recent times, some rather close to the state, others (as *Thomomys*) not within hundreds of miles. Of these, one (*Erethizon*) is of ultimate southern origin but now ranges into the far north and has become adapted to a zone no longer present in Florida. Two (*Ondatra*, *Synaptomys*) are probably of Eurasian or far northern origin. *Ondatra* lives in the southern part of its range in conditions also found in Florida. *Synaptomys* is now confined to zones (Boreal to Upper Austral) not extending into this state. The other genera of this group are probably autochthonous and still occur in zones present in Florida.

Two genera (*Tatu*, *Tagassu*) occur almost directly west of Florida, in some cases under conditions present in this state, and also southward into South America. Two (*Hydrochærus*, *Tapirus*) occur only in the neotropical region in this hemisphere. Of these southern forms, two (*Tatu*, *Hydrochærus*) belong to families of neotropical origin, although they range more widely northward in the Pleistocene, while two (*Tagassu*, *Tapirus*) are of holarctic origin but survive only in the south.

Among rodents, the Pleistocene fauna included most (perhaps all) of those still living, and also three small rodents (a pocket gopher, muskrat and mouse-lemming) ecologically somewhat similar to those still present in Florida and of genera surviving elsewhere. One, the porcupine, was larger, also survives elsewhere, but has no analogue in the modern fauna. Three (giant beaver, two large capybaras) were very large, larger than any rodents in the world to-day, and have no analogues in the modern fauna of Florida or of North America as a whole.

Among carnivores, in addition to recent types, there were three specific variants (a raccoon, a gray fox and a cougar) of genera common in the recent fauna. There were two (a red fox and a coyote) not now very closely paralleled in the Floridian fauna but of genera still com-

mon elsewhere in North America. There were three (short-faced bear, true giant cat, saber-toothed cat) not very closely paralleled in North America now, and on the whole larger and more powerful than any living Floridian carnivores. There were seven species of edentates, referred to three genera of ground-sloths, one of glyptodonts and two of armadillos, which are without any parallel in the modern fauna except for the smaller armadillo which is not unlike some now living but not in Florida.

There were horses and tapirs, three species of each, wholly absent in the Recent fauna of Florida although of genera surviving in very distant regions. There were peccaries in great variety (probably three genera and six species) and three species of camels, all totally unlike any Recent Floridian mammal. There was a bison, different from the modern bison which ranged not far from Florida but not into that state.

Finally there was a mastodon and three mammoths which are also wholly unlike anything in Florida to-day.

The richness of the Pleistocene fauna of Florida in species was extraordinary. It was probably about equal to, and perhaps even greater than, the average variety in single faunas throughout the Tertiary. It was certainly greater than the usual variety in modern faunas of comparable areas.

In view of the marginal, peninsular nature of Florida, its limited size and its relatively uniform physiographic and climatic conditions, this complexity of fauna is all the more surprising. Yet it seems probable that these very factors, which to-day militate against its faunal variety, contributed to this in the Pleistocene.

The outstanding events which led up to the Pliocene and early Pleistocene culmination of mammalian life were a series of world-wide migrations. Interchange of mammals took place between North America and South America on one hand and North America and Eurasia on the other. The result was a continental fauna very

rich in variety and more heterogeneous in origin than most of the earlier faunas. This enriched animal life, in which Florida shared, had probably reached a natural balance by early Pleistocene time.

The outstanding event of the Pleistocene was, of course, the glaciation. The glacial front stood far north of Florida, and its effect on that state was largely indirect but probably profound. Climatic change doubtless occurred, but was probably not great. Hundreds of miles from the glaciers and surrounded, as now, by tempering waters on three sides, in the path of tropical currents, it is not to be supposed that the temperature here was lowered as much as that of the glaciated areas. The isotherms would not be pushed uniformly southward, but rather would be crowded together, producing a steeper temperature gradient between Florida and Canada.

This is largely borne out by the fauna itself. Only one known Pleistocene mammal is of boreal affinities (*Synaptomys australis*), and this is a distinctive species of a genus which does range well into the Upper Austral Zone to-day. On the other hand, a distinct element of the fauna has decided tropical affinities.

The advance of the ice-sheet made a large part of North America almost uninhabitable and another large part inhospitable to most mammals. Southward movement of the mammalian hordes was inevitable. Even aside from the animals which might migrate to great distances, a surging impulsion would be transmitted so that species or individuals which never lived near the glaciated areas would yet be urged southward by the pressure of concentrated populations north of them.

America east of the Rocky Mountains is bifid. An open channel leads into the great expanse of Mexico and hence eventually into South America. Another (and rather less important) route leads into Florida, but this is a blind alley. One must thus envision Florida as a sort of a trap or pound where the animal population

was significantly increased both in number and in variety by the creatures urged on and loosely confined by biotic pressure from the north and with no escape in any other direction. Here in Florida they found an asylum, climatically more favored than almost any other part of the continent, but a scene of the bitter warfare which always follows overpopulation. The favorable conditions here and the absence of the much more severe stress of the northern region probably led to the survival of many species to a much later time than nearer the glaciated areas. There is considerable evidence for this view, quite aside from the theoretical probability of such an occurrence.

Extinction did finally visit the Floridian fauna with results even more dire than elsewhere on the continent. If everything favored variety of fauna in the Pleistocene, there is evidence that the contrary was true after the Pleistocene. Recent Florida is actually less favorable to variety in species than many more continental parts of the United States. Its thirty-three recent species are less numerous than the average for similar areas in this country. Its Pleistocene fauna includes eight genera which survive elsewhere in the United States but are extinct in Florida. Only one Pleistocene genus (*Neofiber*) survives here and not elsewhere,¹ and as yet there is no positive evidence that it ever occurred elsewhere, although this is highly probable. This poverty of the recent fauna is almost as striking in its way as the richness of the Pleistocene fauna. There seems, for instance, to be no purely environmental reason for the absence of the bison in the recent fauna.

CAUSES OF EXTINCTION AND THEIR ACTION IN FLORIDA

The disappearance of a species or large group may be due to any of three causes: transformation into something else, emigration to a different region or racial

¹ It does transgress the political boundaries, but is confined to extreme southeastern United States.

death. In the first case, the group effectually disappears as such but is not really extinct. In the second, it merely changes its location but may be considered in one sense as locally extinct. In the third, unqualified extinction has taken place.

In the present example, it has been shown that the degree of evolution between Melbourne time and Recent was largely or wholly subspecific. No species are known which disappeared because of transformation into other species.

It might be supposed that the presence of genera in the Floridian Pleistocene which survived elsewhere but not there gave evidence of extensive emigration, but this is not true. It is probable that numerous individuals and even whole species did in effect move northward at the close of the Pleistocene and leave Florida, but if so one is at a loss to cite more than one probable example (*Ondatra zibethica*), and this can hardly have been an important factor in modifying the faunas as known. For the most part it is known that these genera did inhabit large areas in the Pleistocene, including much or all of their present ranges and also Florida and other areas outside their present ranges. Where adequately known, the Floridian species of these genera, except *Ondatra*, have proved to be distinct from the living species. These are not examples of emigration but of restriction of range through the true extinction of marginal species. The red fox may be taken as one example. There was a red fox in the Pleistocene of Florida. None lives in the state now, but a red fox still occurs farther north along the Atlantic Coast. The animal did not, however, migrate northward at the close of the Pleistocene. The eastern red fox, *Vulpes fulva*, was already present within its recent range, and the Florida species, *V. palmaria*, became extinct.

The disappearance of so many species from Florida was thus due to their true extinction. The causes of ex-

tion, so much discussed, are manifold. They may be classified roughly as follows:

I. Inherent causes.

a. Independent of environmental change.

1. Disadvantageous specialization.
2. Loss of racial vitality, lowering of reproductive power.

b. Related to environmental change.

1. Highly specific adaptation and loss of adaptability.
2. Lowering of reproductive power through external influence.
3. Special susceptibility to various external influences through size, physiology, habits, etc.

II. Physical environment.

a. Direct.

1. Directly unfavorable climatic and physiographic changes.

b. Indirect.

1. Restriction of range.
2. Physical influence on physiology.
3. Physical influence on biotic environment.

III. Biotic environment.

a. Loss or restriction of food supply.

b. Introduction or multiplication of enemies (including parasites, poisons, etc.).

c. Intensification of competition or introduction of new and able competitors.

'The list is not complete or detailed, but it includes most of the supposed factors of extinction. All are interrelated, and extinction through the operation of a single and simple cause rarely has occurred. The so-called inherent causes are particular: they influence the extinction of certain species rather than others. The environmental causes are general: they influence a fauna as a whole and cause those susceptible for inherent reasons to become extinct.

It has been argued (most recently by Tolmachoff) that the principal if not the only cause of extinction is inherent and independent of external factors. A species or a race is supposed to grow old, as do individuals, to expend its energy, to lose adequate powers of propagation. Two related but really distinct causes are cited:

the development of races with characters which, regardless of uniformity of environment, are actually disadvantageous—the giant Irish deer often given as an example—and the depletion of racial vitality to the impairment of adequate fertility. It is obvious that non-advantageous variations or mutations do occur, but it is an axiom of natural selection that if they are actually disadvantageous and without compensating correlation with other characters they will not be perpetuated to the point of becoming fixed features of a race. The reality of evolutionary momentum or orthogenetic evolution to the point of dangerously disadvantageous specialization with uniformity of other conditions has been challenged and is certainly not proved.

Whatever the case in other faunas, it hardly seems possible to apply this doubtful cause of extinction to the apparently advantageously specialized mammals of Florida. The nature of these extinct mammals also seems to me to be opposed to the theory that senility in itself was a primary factor in this case, whatever may have been true in other instances. The main argument seems to be that animals that were highly specialized and racially old have become extinct, and that external factors, in the opinion of the authors advancing the argument, were inadequate. The analogy with individual life is very precarious if not absolutely false, and the alternative explanation that extinction was due to inadaptability under changing external conditions seems adequate and is applicable to every case of this sort.

Certainly in the specific case of Florida there is much evidence against senility being the main or only cause of extinction and no good evidence for its being a real factor at all. Was *Procyon nanus* depleted of inherent vital force while its larger and equally specialized very close relative and faunal associate *Procyon lotor* was well able to flourish indefinitely? Why were species of *Equus* in North America racially older and less prolific than species apparently almost identical in size and

structure in the Old World? Examples of the insecurity of this assumption could be greatly multiplied from this fauna alone. Two types of animals, the extinct very large-horned bison and the mammoths, might be adduced as evidence for this view. They are not, like some of the other examples, definitely opposed to it, but it seems to me that there is no positive evidence whatever that *Bison latifrons* or *Parelephas columbi*, which became extinct, were in an infertile racial old age while *Bison bison* and *Elephas indicus*, which survived, were racially more productive and vigorous.

The almost simultaneous extinction of two thirds of the Pleistocene fauna can hardly be considered as an inevitable event regardless of external circumstances. The selection of certain species rather than others for extinction must reasonably have been due in most cases or in all to differences between the species, hence to their own characters, but the influence which made these different characters fatal in so many cases must have been external. Aside from other considerations, the mathematical probabilities are enormously against so many and such extremely various animals reaching their period of existence at a time so definite and limited from a geologic point of view, and these probabilities greatly favor the action of some external factors more strongly at this time than at any other.

The problem is dual. What were the external factors that impelled faunal impoverishment? And what were the individual characters that directed extinction to certain species?

The external factors present have already been suggested in part. So far as the evidence is available they were as follows:

I. Physical.

a. Geographic and physiographic.

1. Limited area. Except probably at its extreme southern end Florida was not smaller in Melbourne or post-Melbourne time than now, but this was a small area for the abundance of mammals then present as a result of other causes.

2. Imperfect drainage. There has probably been some in this respect, as there is evidence of slight elevation and new incised drainage lines since Melbourne time.

b. Climatic.

1. Climatic change. In Florida itself the change since Melbourne time has probably been very slight. The average temperature may have been slightly lower, although this is not clear, but it probably has not been significantly higher. There is little evidence bearing on changes in precipitation or humidity.

c. Biotic.

1. Intensification of struggle through enlarged population.
2. Partial duplication of adaptive types through mingling of species of different geographic origin.
3. Restriction of food supply. There is no evidence of the restriction of plant food beyond that probably present in Melbourne time, and now present, which is nearly a maximum. The extinction of numerous herbivores would constitute a very decided restriction of carnivorous food supply.
4. Introduction of new enemies. The introduction of new parasites or pathogenetic organisms or of poisonous plants is a possibility on which there is no evidence. Within the fauna itself there are no enemies, with one exception, that had not been present for a considerable time and hence were not probably in faunal equilibrium. The important exception is man, most powerful and destructive of all vertebrates. There is now good evidence that he was not present much before Melbourne time, but did appear just before the most marked faunal impoverishment.
5. Indirect influence of faunal disturbance elsewhere on the continent. There is evidence that many of the groups surviving in Florida in Melbourne time had previously become locally extinct over large areas. This would have a marked local influence in the diminution of herds, in lessened opportunities for cross-breeding, in the liability to total extinction through purely local or temporary causes, in the intensification of struggle with enemies and possibly in other ways.

These circumstances, acting together, indicate the reasons for extinction of so many animals at this particular time. All animals were undergoing stress which resulted in the elimination of those most definitely affected.

The intricately correlated and in part unknown factors which led to the extinction of some species rather than others can not be fully elucidated, but are suggested to some degree by their characters.

The only case in which climatic change can be considered as a definitely possible factor is that of *Synaptomys*. Even in this case climate was probably secondary to other causes. In Florida this animal was in competition with numerous small cricetines and microtines and preyed upon by numerous carnivorous vertebrates. Its survival to the northward may be due to its adaptation to particular and specialized conditions, giving it sufficient advantage in competition and sufficient protection from enemies to permit survival. They are obscure and, usually, relatively rare animals, living principally in a cold moist habitat, in the southern part of their range almost wholly in cold sphagnum bogs. Similar habitats may or may not have occurred in Florida in Melbourne time, but do not occur there now.

Certain of the Pleistocene species of Florida represented essential duplication of adaptation. The clearest examples are the association there of *Thomomys* and *Geomys* and of *Ondatra* and *Neofiber*. These genera all survived to the recent, but *Thomomys* is not now found with *Geomys*, nor do *Ondatra* and *Neofiber* occur in the same area now. Just what subtle local advantage decreed that in Florida *Geomys* and *Neofiber* should be successful is not clear. Equally striking and similar in significance are apparent cases of the existence of two closely related species of one genus where but one is now present: *Procyon* with the Pleistocene species *U. cinereo-argenteus* and *U. seminolensis*, of which only the former survives, and *Procyon* with *P. lotor* and *P. nanus*, only *P. lotor* surviving. The cougars are not yet well known, but it is possible that they too included two closely related species, and only one, *Felis coryi*, has survived.

Then there is a duplication less complete and probably to be considered rather as a contributory than as a pri-

mary cause of extinction. *Arctodus floridanus* belongs here. Its mode of life must have resembled that of *Euarctos floridanus* somewhat, but not to the point of truly lethal competition. The same would be true of *Ænocyon ayersi* with respect to *Canis floridanus*, if indeed the latter wolf was present in the Pleistocene. *Vulpes palmaria* and *Canis (Thos) riviveronis* doubtless met competition to some degree from the gray fox, wolf and other carnivores, although they are well able to survive such competition elsewhere.

The examples just given suggest a crowding into Florida of species which would not ordinarily develop in such a limited area and probably would not indefinitely remain distinct there together. These abnormal conditions were probably due to the peninsular nature and the position of Florida and to the impulsion of unfavorable climatic and other conditions in the north.

The decisive influence in the extinction of the larger predaceous carnivores, *Canis (Ænocyon) ayersi*, *Felis veronis* and *Smilodon floridanus*, was probably secondary: the failure of adequate food supply for which they were specifically adapted, through the extinction of most of the larger herbivores. Temporary diminution of plant food during a season or two may have further jeopardized herbivores already in a precarious position due to other causes, but this is purely speculative. There is no evidence of any general or long-continued diminution of their food supply.

There remains a large part of the fauna probably not much affected by these more obvious partial causes. Among rodents, the giant beaver and capybaras belong here; among edentates, all the known forms, ground-sloths, armadillos and glyptodonts, and among ungulates, the horses, tapirs, peccaries, camels, bison, mastodon and mammoths. Their extinction is particularly striking and puzzling and was probably due to causes still more complex than those affecting the other mammals. Unlike the species so far discussed, they apparently could live

in Florida under recent conditions, and yet there are no mammals there which parallel them at all closely in adaptation.

These diverse animals do possess some important features in common. All are herbivorous. All are large, the white-tailed deer being the only living herbivore as large as any of them, and the smallest, *Tatu bellus*, a large armadillo twice the size of the species living in the Southwest. With the probable exception of the edentates, all are communal or even highly gregarious. Most of them apparently thrived best in a rather warm climate and some were definitely subtropical. The rodents are water-loving forms, but the others are relatively intolerant of swampy or very wet situations. All are unusually attractive prey to carnivores, including man, and many of them are attractive to primitive man for special reasons, such as desirability of their hides, pelts or teeth. Most of these characters can be shown to have had a possible influence in the extinction of these groups.

Large size exposes its possessors to especial danger in times of stress. Large animals multiply more slowly, have a longer juvenile period, require more food and are relatively fewer in number in a given area.

Communal or herd life is somewhat analogous to large size in that it is especially advantageous under normal conditions but may become peculiarly disadvantageous under abnormal conditions. In some cases the safety of the individual, especially when young, is dependent on the functioning of the herd. In such cases a temporary decrease in herd size may initiate extinction while an equal temporary diminution in the number of individuals of the solitary species would have no permanent effect. Communal or gregarious animals are much more susceptible to epidemics. Restriction of range or separation of groups is more likely to lead to degeneration. With the vast inland region populated by similar and accessible groups, the vitality of the species in Florida would

be maintained by intermigration and marginal contact. With the removal of this background ~~and~~ with loss of contact with it, as seems definitely to have occurred at the end of the Pleistocene, the distinctly limited Floridian herds were thrown on their own resources with degeneration as an almost inevitable result. Finally, animals that live in groups are especially subject to the depredations of man as seen in the methods of mass slaughter known even to early man and also to the disruption of the necessary communal life by the killing of leaders or males.

Various factors may be considered as possibilities or contributing causes of perhaps minor import. The introduction of new parasites or diseases or poisons is distinctly possible, although hardly a complete or general explanation in view of the diversity of the animals affected. Poor drainage and unusually wet seasons may have had an indirect influence in the still greater restrictions imposed on available range and possibilities of free migration. Change in average temperature was probably not great, but in these particular animals even a short succession of unusually severe winters might be very dangerous to the race.

The groups which did so dramatically and, it would seem, so mysteriously become extinct were on the whole just those in which, under the conditions at this time and in this area, the combined action of various adverse circumstances might lead to a crucial diminution in numbers. In animals of different character (as those that did survive in Florida) or in similar animals under different conditions or not subjected to this combination of stresses (as close relatives that survived in other regions) this diminution might be purely temporary. These particular groups were unduly susceptible to permanent effects from temporary causes.

SHORTER ARTICLES AND DISCUSSION

SPERMATOPHORES OF AN OREGON CRAYFISH

Of the seventy or more sorts of crayfish found in North America almost all are of the genus *Cambarus* and only a few are of the genus *Potamobius* (commonly known as *Astacus*) and these are restricted to the western part, while *Cambarus* occupies the area of the Mississippi and easterly as well as Mexico and lower Canada.

While the genus *Cambarus* is peculiar to this region, the genus *Potamobius* has several species in Europe and in western Asia where no other genera are found. In the east of Asia are some crayfish referred to a genus *Cambaroides* that has some resemblances to each of the other two genera, *Potamobius* and *Cambarus*. All the crayfish known south of the equator are of many genera differing from these north of the equator.

Underlying any adequate explanation of the peculiarities of this geographical distribution should be a better understanding of the differences between these several genera and species.

As far as known, *Potamobius* differs from *Cambarus* internally in valvular arrangement of the heart and in slight numerical diversities in gills. Externally, however, there are marked differences in the secondary reproductive organs, because in the male *Potamobius* has more simple and vaguely elaborated organs for transferring the sperm as compared with the highly specialized and perfected organs of *Cambarus* that differ markedly in different species. Moreover, the female *Potamobius* is generalized as having no organ into which the sperm may be received, while the female of *Cambarus* has in each species a differently fashioned specialized external pocket in the shell into which the sperm is placed by elaborate, strenuous and precisely adjusted exertions of the male.

It is now well known that the "annulus ventralis," formerly known only as a structure relied on as a specific character in *Cambarus*, is the sperm receptacle peculiar to *Cambarus* and not found elsewhere, though an analogous structure occurs in the common American lobster.

Huxley and others described the sperm transfer in the genus *Potamobius* of Europe in which there is no receptacle and the sperm is deposited in capsules or spermatophores on the parts of the shell of the female over which the eggs will travel upon being

laid, when the sperms are supposed to emerge and to become attached to the eggs.

As was to be expected, we find this same diffuse application of spermatophores to take place in some American crayfish of this genus *Potamobius*. In 1927, some forty living and forty dead *Potamobius trowbridgei* received from Professor Lawrence E. Griffin, of Reed College, Oregon, were apparently in breeding condition at that season, November, since two dead females had still some few eggs attached to the pleopods, and one dead male spermatophores attached to the ends of the first stylets.

After a few days in captivity one of the females was found dead with conspicuous white spermatophores attached to the ventral surface. The spermatophores were slender white threads of variable lengths, but an average might be about five millimeters long and about $\frac{1}{4}$ mm thick. They were attached chiefly in two dense masses, but with scattering exceptions as follows. On the bases of the large antennae there were three on the right and five on the left, eight on the head in all. Just anterior to the oviduct openings on the thoracic surface depressed below the legs there were two small spermatophores on the right and one on the left; these eight plus three anterior to the oviducts would be of no use as they would not have access to the eggs which pass posteriorly from the oviducts to the abdominal pleopods. Just posterior to the oviduct openings in the depressed thoracic surface behind the leg base, there was one spermatophore on the right and one on the left and on the median sternum between the fourth legs there were four.

Coming now to the main masses: On the sternum between the fourth and fifth legs there was a large mass made by adhesion of thirty-three spermatophores, twenty in the main central mass with five on the right between the leg bases and eight on the left between the leg bases. All these presented distinct tips that were counted while the attached bases were stuck together more or less indistinguishably.

On the movable sternum of the thorax just anterior to the first abdominal pleopods there was a second large mass of spermatophores, forty-one in all, thirteen on the right, twenty-five on the left and thirteen on the middle. Thus the entire number of spermatophores counted was ninety-one, and of these all but eleven were placed posterior to the oviduct openings and hence could have been of use if their sperms issued properly to connect with the eggs.

How long these spermatophores retain the sperm and by what means they are emptied for the fertilization of the eggs is not directly known. The suggestion was made by Lenckart that by the gradual change of the wall of the spermatophore, (a secretion from the lining cells of the deferent duct), a discharge of sperms might be caused; but this would need be accurately timed. Very likely, as surmised by Meyer, it is the action of some secretion of the female at the time of laying eggs which induces the emptying of the spermatophores. In any case there may well be considerable loss of sperm. From this point of view it is of interest to determine approximately the number of sperms available in all these spermatophores.

Previously it had been computed for *Cambarus limosus* that the numbers of sperms deposited in the annulus ventralis or sperm receptacle was in the neighborhood of 60,000 to 100,000, and that after laying some 10,000 of these might still remain unused in the inmost recess of the receptacle. As the female lays but 200 to 600 eggs at once, according to age and size, there is here ample provision for each egg to become fertilized and in fact it was seldom, in captivity, that all the eggs were not fertilized. In the male the length of the deferent duct was 60 mm, and it might contain on both sides 2,000,000 sperms, sufficient to fertilize the eggs of many females.

We found the deferent duct in *Potamobius trowbridgei* longer, as might be expected in this larger animal, but disproportionately longer, some 230 mm, and thus the male may well supply more sperms than is the case in the above *Cambarus*.

The number of sperms actually found upon the above female *Potamobius* was estimated to be one half million as follows. In an average spermatophore teased into bits in drops of water and then dried on glass, stained with Haidenhain's haematoxylin, the sperms were counted as 5,412. In water the sperms previously densely compacted in spheroidal form expand the ray-like appendages and glide out along the glass and thus they may be counted. The rays were very long, about twenty, but apparently variable in number and thus much more numerous than yet observed in *Cambarus*.

Assuming that these findings recur again in other species of *Potamobius* and of *Cambarus*, we may tentatively add to the other generic differences differences in lengths of deferent ducts, differences in numbers of sperm arms, and differences in numbers of sperms supplied at one time by the male.

With increasing knowledge of the generic differences it becomes increasingly difficult to picture just how such differences may have arisen; as modifications of some common basis; or derivation of one genus from the other, since geographical distribution leaves little doubt that *Cambarus* is the newest genus of crayfish.

The existence of a sperm receptacle in *Cambarus* is apparently correlated with an economy of sperms, since the numbers used for one egg-laying are much smaller than in *Potamobius*, where the spermatophores are scattered and not concentrated within a small receptacle; moreover, the central position of the receptacle seems better to assure the contact of the sperm with the issuing eggs than the wide spreading of the spermatophores on the outside of the shell, since the eggs tend to glide along the middle line, though emerging right and left.

It is then possible to regard the invention of the sperm receptacle as a gain in economy and a useful improvement that might, conceivably, be of some moment to the race; hence natural selection might have had a hand in its preservation.

In brief, the accuracy of aim in sperm transfer may have enabled *Cambarus* to avoid some of the loss of materials caused by the scattering methods of *Potamobius*. Other characters of these two genera, such as the slight differences in numbers of gills and the numbers of arms of the sperms, can scarcely be thought of as in themselves of any survival value, and raise the question whether we shall regard this whole complex of secondary sexual organs and their functions as arising from unknown variations in supposed genes producing results in part seemingly significantly useful.

Pending experimentation we remain quite ignorant of any explanation of the causes for such generic differences as those separating *Potamobius* and *Cambarus*.

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CHROMONEMATA IN SOMATIC AND MEIOTIC MITOSES

STUDIES of root-tip mitoses of *Allium cepa* and of *Iris versicolor* reveal chromosome structure and chromonemata behavior essentially similar to that previously described for somatic mitoses of *Tradescantia pilosa* (Kaufmann, 1926a) and *Podophyllum peltatum* (Kaufmann, 1926b). Sharp also has reported the presence of chromonemata in the various phases of root-tip mitoses of *Allium cepa*, as part of a more extensive investigation

of large somatic chromosomes (Sharp, 1929). In my studies of *Tradescantia* and *Podophyllum*, the anaphase chromosomes were found to be composed of two visibly dissimilar substances, designated as chromatic and achromatic. The chromatic material exists in the form of a pair of spiral threads, embedded in the achromatic matrix. The threads or chromonemata persist through the telophases and the interphases, although discernible with difficulty in the latter stages, on account of the building of interchromosomal anastomoses. In the succeeding prophase each of the parallel threads was observed to give rise to two new chromonemata, which are destined to separate at the second succeeding anaphase. Metaphase chromosomes of somatic mitoses are therefore tetrad, with respect to the chromatic material, since each half chromosome contains a pair of chromonemata.

The race of *Iris versicolor* used has a diploid chromosome number of about sixty-eight. This proved good material for the study of chromonemata in smaller chromosomes. Chromonemata were sought also in the small chromosomes of the root-tips of the ferns *Polypodium incanum* and *Adiantum pedatum*. In these it is possible to identify the spirals in protruding ends at anaphases, and occasionally to recognize duality during these stages and the telophases. Late prophase gives some indication of the spirals within each of the half chromosomes. Up to the present it has been impossible, however, to determine either the time of origin of the chromonemata, or their behavior during the phases of mitosis not mentioned. Fragmentary evidence of detached phases is suggestive, nevertheless, of the organization more readily discernible in larger chromosomes throughout the mitosis.

The behavior of the chromonemata in meiosis is the particular concern of the present preliminary report. The following account is based primarily on observations of microsporogenesis in *Tradescantia pilosa* and *Rhoeo discolor*, although the chromonemata have been traced through meiosis in the additional species, *Podophyllum peltatum*, *Iris versicolor*, *Pinus echinata*, *P. palustris* and *P. taeda*, and have been seen at metaphase of the first division in *Allium neopolitanum*, *Gasteria* sp., and *Tradescantia virginiana*. The occurrence of a "bouquet stage" in *Tradescantia* and *Rhoeo* has been reported (Kaufmann, 1925), and the tetrads of the first maturation division of these two species have been figured.

At the anaphases and the telophases of the last pre-meiotic division the chromosomes exhibit the parallel chromonemata, as reported in an earlier paper (Kaufmann, 1926a). The chromonemata persist through the subsequent interphases, although the space between them often is obscured. That duality exists is indicated by the split along the turns of the coil occasionally seen in leptotene-threads. In *Tradescantia*, synchronous with the extension of the leptotene-threads, the plasmosomes flatten against the inner face of the karyotheca. There they form an "attachment plate" for the elongating threads, which accordingly are polarized, and loop out into the nuclear cavity initiating the "bouquet stage." Synzesis, seen in some preparations of this stage, can be controlled by proper fixation.

At regions of maximum extension the leptotene-threads are smooth, except for such chromomere-like swellings as are due to the tightening of the coils incident to the elongation of the threads. The appearance of a spireme composed of discrete particles or chromomeres suspended on a lighter staining thread was encountered, but better preparations of these stages showed only nodal points caused by sharp turns of the coils. In many preparations it is apparent that complete elongation of the homologous threads is not always prerequisite to their pairing. Nuclei in the amphitene-condition evidence this most strikingly, the coils appearing with diagrammatic clarity both in the paired and the unpaired portions of the chromosomes. This observation lends the strongest support to the parasynaptic interpretation, for it is possible sometimes to determine that pairing is side-by-side between chromosomes which are longitudinally split (a split referable to the prophase of the last pre-meiotic mitosis.) Verification of parasynapsis is of especial interest in *Tradescantia* and *Rhago* because of the ring or chain formation, most strikingly seen at metaphase, and involving some or all of the bivalents. The observations here reported support the conclusions of other workers that parasynapsis is tenable with subsequent end-to-end linkage.

During the ensuing prophase stages the chromonemata become increasingly conspicuous, concurrent with chromosome shortening and condensation. At metaphase each homologue of the bivalent contains two spirally arranged, parallel, chromatic threads embedded in an achromatic matrix.

As the anaphase chromosomes move toward the poles (or slightly earlier) the chromonemata separate, the achromatic

matrix splitting simultaneously. The majority of preparations of these stages reveal a single spiral thread within each member of the dyad, but occasional smears show duality. That the latter is probably the true structural condition is indicated at the time of telophasic transformation, when dissolution of the achromatic matrix exposes more fully the two chromonematic skeletons which are destined to separate at the first post-meiotic anaphase.

During interkinesis the chromonemata may be obscured temporarily by anastomosing. Prophase and metaphase chromosomes of the second division, even at the time of maximum extension, show pronounced internal spirals. Such chromonemata can be traced through the succeeding anaphases and telophases.

Diagrams illustrating the above interpretation of somatic and meiotic mitosis are inserted as Figs. 1 and 2.

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A MODIFIER OF PIEBALD SPOTTING IN MICE¹

THE F₂ offspring of a cross between a strain of black-eyed-white mice and a self-colored strain exhibited a great variety of spotting patterns. Practically all the spotted F₂ animals were tested for the black-eyed white gene by mating them to animals known to contain that gene. The production of anemic offspring (WW animals, cf. De Aberle,² 1925) by the tested animal immediately identified it as a carrier of the black-eyed white gene. By this process there were identified also a number of spotted animals that did not carry the gene but were ww in constitution. A few of these proved to be wwSs in constitution because when mated together they produced a number of self-colored animals. Furthermore the spotting of such animals was very slight indeed, being confined to a few white hairs on the forehead or belly and slight white spotting on the toes and tip of the tail. The wwss

¹ From the Bussey Institution, Harvard University, Forest Hills, Boston, Massachusetts.

² S. B. De Aberle, *AMER. NAT.*, 59, 327-335. 1925.

animals proved to be easily separable into two classes. The class called dark-faced was characterized by practically no face spotting, definite white spotting on toes and tail and a varying amount of belly spotting. In this class the maximum amount of face spotting consisted of a small forehead blaze which might occasionally extend in a narrow white line to the nose. The "belt" of white spotting observed in certain strains of guinea mice was not definitely present in these animals though in certain animals a slight amount of dorsal white was observed in the position of the "belt." The second class resembled the first in all details except that it contained definitely white-faced animals. The white face was the type commonly observed consisting of a V-shaped area of white hairs with the point of the V located between the ears. In a number of the animals, small, irregularly distributed areas of dark-colored hairs were located on the white face. In a few cases the distinction was not completely clear between a somewhat extended forehead blaze and a white face with a large number of dark areas. It was decided, therefore, to undertake a certain amount of inbreeding in an attempt to obtain clearly contrasting types.

As soon as the inbreeding was begun it became evident that white-faced animals bred true with remarkable consistency. After about four generations of brother-sister matings of white faced by white faced, 317 animals were produced in 76 litters and 314 of these were definitely white-faced. The three exceptional individuals were derived from parents which possessed much reduced face spotting that might have represented rather large forehead blazing, and none of the three was completely dark-faced.

The matings of dark-faced animals *inter se* fell into two classes: (1) those which produced both dark-faced and white-faced offspring; (2) those which produced only dark-faced offspring. After about four generations of brother-sister mating 58 litters of class (2) were produced containing 272 dark-faced young. Concurrently there were produced twenty-six litters of class (1), 127 animals of which were dark-faced and 49 of which were white-faced. On the expectation of a 3 to 1 segregation of dark-faced from white-faced we should have 132 dark-faced to 44 white-faced; the deviation is 5, the probable error, 3.87.

Furthermore, certain of the matings consistently involved the breeding of white-faced brothers to dark-faced sisters and *vice versa*. In 47 litters from such matings 253 young

duced, of which 129 were dark-faced and 124 light-faced. The deviation from a 1 to 1 ratio is 2.5, the probable error, 4.65.

It was, therefore, concluded that white-faced spotting was due to a single recessive modifier independent of the gene for piebald spotting (*ss*) but exerting its effect only in the presence of the piebald gene. This recessive modifying gene may be called *l*. White-faced piebalds are therefore *ll ss*, and dark-faced piebalds either *Ll ss* or *LL ss*.

It might be objected that the 1 to 1 ratio obtained for matings of white-faced piebalds to dark-faced piebalds is misleading, inasmuch as certain of the dark-faced animals might have been *LL ss* in constitution, and therefore an excess of dark-faced animals should be expected. It must be remembered, however, that the data for these crosses were derived from consistent brother-sister matings of white-faced animals to dark-faced sibs, so that one might expect an excess of dark-faced young only in the first brother-sister generation. Most of these data are from the 2nd, 3d and 4th brother-sister generations. Similarly, certain of the dark-faced animals in the group of dark-faced matings producing only dark-faced young must have been *Ll* in constitution. Certain of these animals did, in fact, prove to be so when they were tested in subsequent generations, producing approximately equal numbers of white-faced and dark-faced young when mated to white-faced animals. By far the larger number, as was to be expected, proved to be homozygous *LL* when crossed to white-faced animals since all the offspring (103 animals) were dark-faced.

It was decided, after four generations of brother-sister matings, to undertake an independent test of the existence of the gene *l*. Accordingly two types of matings were made: (1) animals from lines of true breeding white-face were mated to self-colored dilute brown mice of the long inbred Little strain; (2) animals of true breeding dark-faced lines were also mated to self-colored dilute brown. F_2 , F_3 and backcross progeny were obtained. The results are given in Table I. The dark-faced animals used showed a large amount of belly-spotting. They were easily differentiated from the few F_1 animals that showed a slight amount of white spotting. Most of the F_1 animals were completely self-colored; a few exhibited slight forehead blazes and occasional white spotting on the toes and tail. Such animals appearing in F_2 were classified as self-colored. There may have been some overlapping between these and very dark spotted

dark-faced animals, but this is doubtful inasmuch as practically all dark-faced F_2 animals showed large belly spots often accompanied by dorsal spotting in the position of the "belt."

It is evident from these data that white-faced mice are homozygous for a gene (ll) which, in the presence of the normal spotting gene (ss), extends white spotting to the face.

Final proof of the existence of this gene was had by inbreeding white-faced F_2 and backcross progeny of these matings. Such animals were bred brother to sister for ten generations. They always produced white-faced young. Furthermore, subsequent matings of dark-faced piebalds *inter se* were of two types, those producing only dark-faced piebalds and those producing approximately three dark-faced piebalds to one white-faced.

TABLE I

Generation	Self-colored		Dark-faced		White-faced		Deviation	Probable error
	obs.	exp.	obs.	exp.	obs.	exp.		
F_2 from (F_1 dbr $\times$ white-face)	321	325.5	78	81.37	35	27.13	(3:1)4.50 (15:1)7.87	6.08 3.40
F_2 from (F_1 dbr $\times$ dark-face)	95	92.25	28	30.75	0	0		2.75 3.24
Backcross, inbred white-face $\times F_1$ (dbr $\times$ white-face)	53	50.50	26	25.25	22	25.25		2.50 2.94
Backcross, F_1 white-face $\times F_1$ (dbr $\times$ white-face)	18	22	11	11	15	11		4.00 1.94
Backcross, inbred dark-face $\times F_1$ (dbr $\times$ dark-face)	53	47	41	47	0	0		6.00 3.25
* F_2 from (F_1 dark-face mated <i>inter se</i> [from F_1 dbr $\times$ white-face])	0	0	71	73.50	27	24.50		2.50 3.34

* From matings producing at least one white-face animal.

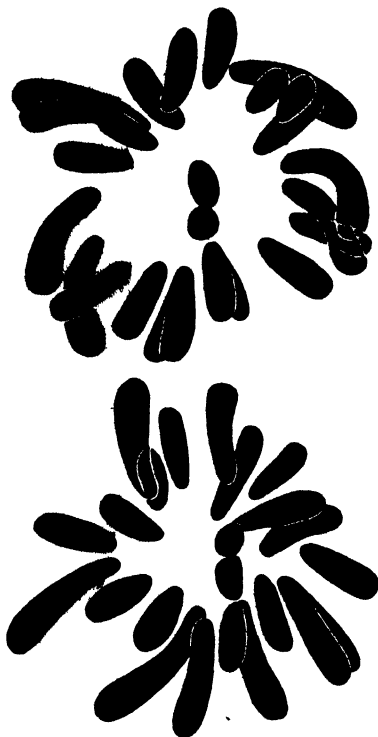
Efforts were made to obtain by inbreeding extremely uniform strains of dark-faced and white-faced piebalds. After four years of inbreeding most of the strains were lost. It is hoped, however, that sufficient satisfactory material remains to permit a further examination of certain variations associated with piebald spotting.

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THE CHROMOSOMES OF THE SOUTH AMERICAN
OPOSSUM, *DIDELPHIS PARAGUAYENSIS*

UP to the present time no cytological data have been available concerning the chromosome complex of the South American opossum, *D. paraguayensis*, notwithstanding the theoretical interest which would be aroused should the chromosome number and morphology prove to be characteristic of the genus, instead of



being limited to the North American species, *D. virginianus*, investigated by (1) Jordan, (2) Painter, and (3) Hoy and George, and also should it be established that the sex chromosomes correspond to the X-Y type.

In the testis of the young opossum there is an abundance of gonial cells which show the chromosomes clearly. One finds without great difficulty eleven pairs of chromosomes oriented radially in the equatorial plate, among which the different sizes are clearly distinguished. The characteristic shape of the elements is that of a telomitic rod. There are eight small pairs, the

smallest being constituted of two unequal ~~chromosomes~~, the sex chromosomes. The remaining three large pairs of elements are of almost equal length, although one of them is frequently disproportionately large. As observed by other authors in different genera of marsupials, the sex pair is usually located in the center of the spindle.

Hoy and George found that in some equatorial plates there were sometimes, in addition to the chromosomes X and Y, one or two elements occupying the center of the plate in the same position, but, at least in the cells observed, we have not been able to encounter this arrangement which may possibly be due to a technical artifact.

We agree perfectly with the seriation ~~observed~~ by Hoy and George in the somatic complexes of different tissues of *Didelphis virginiana*. In the same way *D. paraguayensis* has also three large pairs, seven medians, and the sex pair X-Y.

MUSEO DE LA PLATA.

LA PLATA, ARGENTINA

FRANCISCO A. SAEZ

A METHOD FOR CLEARING LEAVES

THE following method has proved quite effective for clearing and decolorizing whole leaves darkened by preservatives and containing considerable quantities of tannin.

The leaves to be cleared are placed in test tube and covered with a saturated solution of chloral hydrate for 48 hours or longer. The chloral hydrate is then poured from the material and enough potassium chlorate added to fill the bottom of the test tube to a depth of about $\frac{1}{2}$ inch. The material is then covered with concentrated nitric acid and let stand until the leaves begin to change color. From 10 to 30 minutes is usually sufficient. The nitric acid is then poured out, leaving the potassium chlorate and leaves in the test tube, and the material again covered with a saturated solution of chloral hydrate. In this solution there will be a continuous liberation of chlorine gas and within less than a week the leaves should be quite transparent. If necessary the nitric acid treatment may be repeated.

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NOTES ON DIFFERENTIAL GROWTH

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IN reviewing the literature of differential growth in animals, I have come across several papers containing data whose analysis has yielded results bearing on the subject. As the authors of the papers have not considered the bearings of their facts on differential growth, I am summarizing them here.

PART 1. GROWTH-GRADIENT IN THE ABDOMEN OF CRABS

A paper by D. Atkins (1926), gives scale drawings of different stages of the female pea-crab (*Pinnotheres pisum*). I have taken measurements of her drawings and reduced them to absolute figures in accordance with the magnifications she gives. The result is given in Table I.

Owing to the convexity downwards of the abdomen, which increases rapidly in later stages, it is probable that the measurements of segments are sometimes too small. This will apply very slightly to the length of the segments towards the tip, considerably to those near the base (nos. 4 and 3). It will apply also to the breadth-measurements in later stages, especially of the broader segments. This is, however, not likely to make more than 10 per cent. difference to the measurements.

To give us an idea of the differential growth of different parts, we can take the ratios of the measurements of the last stage with complete measurements (stage 5; ♀ 7) to those of the smallest stage (stage 1; ♀ 1); this will give

TABLE I
Pinnotheres pisum. ALL MEASUREMENTS IN MM.

	♂	♀ 1	♀ 2	♀ 3	♀ 4	♀ 5	♀ 6	♀ 7	♀ 8
Carapace width...	5.98	2.10	4.90	4.44	5.98	6.22	8.72	10.14	about
Carapace length	6.06	[2.25]*	5.06	4.44	5.92	6.14	8.30	9.30	14.
Abdomen length	3.58	1.406	3.02	2.94	3.96	4.54	6.92	9.10	
Length of separate segments of abdomen									
... 3	.706	.369	.48	.38		.46			
... 4	.623	.264	.52	.54	.74	.92	1.24	1.66	
... 5	.670	.223	.76	.48	1.00	1.00	1.90	2.22	1.80
... 6	.670	.230	.60	.66	1.06	.82	1.92	2.28	2.87
Telson	7 .777	.176	.48	.76	.86	.92	1.34	2.04	2.77
Breadth of separate segments of abdomen									
... 2		.845							
... 3	1.920	.697	1.70	2.52	5.00	4.98	7.60	9.4	
... 4	1.575	.553	1.54	2.74	5.62	4.24	8.34	10.92	15.35
... 5	1.270	.459	1.26	2.76	5.72	4.10	8.48	10.80	15.46
... 6	1.035	.405	1.06	2.50	5.21	3.75	7.38	10.00	14.63
Telson	7 .800	.257	.70	1.84	3.56	2.50†	5.30	7.30	11.19
	Stage 1	Stage 1	Stage 2	Stage 3a	Stage 3b	Stage 4	Stage 5	Stage	

* The length can not be measured, as the upper surface is not figured. It may be taken as 2.25.

† The last three measurements are only approximate, owing to the overlap of the limbs in the drawings.

a measure of the increases of the different parts. We can further compare the rate of other parts relative to that of

TABLE II
 RELATIVE GROWTH OF PARTS OF ABDOMEN IN *Pinnotheres*

	car.br. car.l.		abdomen segments l.				abdomen segments br.			
			4	5	6	7	3	4	5	6
Increases of parts (ratio of measurements) stage 5: stage 1	4.83	4.13	6.29	9.96	9.92	11.59	13.49	19.75	23.55	24.7 28.4
Ratio of increases to increase of carapace width	1.00	0.86	1.30	2.06	2.05	2.40	2.79	4.09	4.88	5.12 5.98
Growth coefficients (relative to carapace length)	1.11		1.30	1.62	1.62	1.73	1.83	2.10	2.23	2.26 2.36

carapace-breadth by taking the increase of the latter during this period as unity and reducing the other increases (ratio, stage 5; stage 1) proportionately.

For comparison with other forms, however, we can obtain an approximate value of the growth-coefficient (k) from the formula

$$k = \frac{\log y_1 - \log y_0}{\log x_1 - \log x_0}.$$

x is here taken as carapace *length*, x_1 at stage 7, x_0 at stage 1. The figures for k are given in the last line of the table.

Before considering the abdomen, we may note that the carapace begins longer than broad, but owing to the higher breadth-growth its proportions are finally reversed. In the abdomen, the first thing to notice is the extremely high relative growth in width of certain segments, up to nearly six times as fast as the increase of the carapace. These are among the highest figures yet found for differential growth.

More interesting, however, is the demonstration of a growth-center and growth-gradient. In previous papers, (Huxley 1927, etc.) I have shown that where an organ is growing more rapidly than the body (heterogonic growth) it appears never to be growing uniformly throughout its length, but to have a growth-center where growth is most intense, with the intensities of growth grading down steadily in neighboring parts, forming a growth-gradient, single if the growth-center is terminal in the organ, double if it is not terminal. In markedly heterogonic organs, the growth-center appears to be usually terminal or sub-terminal, in slightly heterogonic organs, central or nearer the base.

In this case, the growth-center is clearly terminal (if we consider the segments as units, which is all we can do). This is true both for length and for breadth; but the gradient is much steeper for breadth.

This is hardly what inspection of the drawings would suggest, since the central parts of the abdomen (seg-

ments 4 and 5) though not broadest in the youngest females, become the broadest in the old females, being much broader than segment 7. They achieve this because, although their rate of increase is less, their initial breadth was correspondingly greater.

When we take the ratio of the relative growth in breadth to that in length, as measured by the ratio of the growth-coefficients, for the separate segments of the abdomen, we find the following result. Segment No. 4, 1.62; No. 5, 1.46; No. 6, 1.40; No. 7, 1.36. There is thus a steady decrease, indicating that between breadth-growth and length-growth there exists an orderly relation. Although the last segment is growing the most rapidly in breadth, and although its breadth-growth is much higher than its length-growth, it is deforming itself in the transverse dimension less rapidly than are the more proximal segments. Thus here we have the growth-gradient for length steeper than that for breadth, although at a lower absolute level.

A paper by Sasaki (1928) makes similar measurements for a Japanese shore crab (*Telmessus cheiragonus*), both for males and females. He finds that the abdominal segments grow according to the simple heterogony formula $g = bx^k$, where y in the organ studied, x a standard part (here the carapace length), and b and k are constants. The value of k of course gives the ratio of the growth of the part to that of the standard. We may conveniently tabulate all the values of k for the breadth of the several abdominal segment of both sexes and also for total abdominal length; (the range covered is carapace length 11 to 77 mm).

TABLE III
TELMESSUS CHEIROGONUS: GROWTH RATIOS IN THE ABDOMEN

Breadth of segments	2	3	4	5	6	7	Total length
♂	1.10	1.10	1.14	1.14	1.10	1.00	1.06
♀	1.19	1.23	1.32	1.34	1.36	1.15	1.11

The lengths of the separate segments were unfortunately not taken. In *Telmessus* the carapace width increases less rapidly than the carapace length ($k = 0.95$). If the increase between smallest and largest specimens be taken, and the ratio of the increase of the most rapidly broadening abdominal segment to that of the carapace length be calculated, we find it is very small in comparison with the value for *Pinnotheres*: 1.9 for the 6th segment of *Telmessus* as against 5.88 for the 7th segment of *Pinnotheres*. (In the male, the highest similar ratio is 1.45 for the 5th segment.)

Thus in both sexes of *Telmessus* there exist growth-gradients in the abdomen. That of the male is not at all steep, and has its center in the 4th and 5th segments; that of the female is twice as steep, and has its center in the penultimate segment.

It is interesting to find the steeper female growth-gradient with its center more terminal than that of the male, but less terminal than the extremely steep female gradient in *Pinnotheres*.

There remains one point to be discussed. Atkins describes two forms of females in stage 3, one with relatively long and narrow abdomen (represented by ♀ No. 5), the other with shorter and broader abdomen (represented by ♀ No. 4). So far as one can gather from the few specimens available, the development of the female abdomen is somewhat as follows. Several moults may take place during stage 1, the carapace-width of which ranges from 2.1 to about 5.0 mm (Atkins gives 4.9 mm as the maximum, but her Fig. 3, according to the magnification given, is 5.06 mm in carapace-width). During this phase, there is relatively little differential growth within the abdomen. Atkins states that there is *no* change of form, but a comparison of her small and large crab of this stage (♀ 1 and ♀ 2) indicates that this is not strictly accurate: the differential growth of the segments has already begun, the ratio of their breadth-increase to that of the carapace ranging from 1.05 (3rd segment) to

1.13-1.19 (other segments). The measurement-errors are here so relatively large that it is unwise to try to establish any gradient in the segments.

The onset of rapid differential growth may begin at very various absolute sizes. If it takes place when the animal is of about $3\frac{1}{2}$ mm carapace width we should (following Przibram's rule of the approximate doubling of volume of Crustacea at each moult) at the next moult get a crab of about the size of our ♀ 3. If, on the other hand, the onset of this phase is delayed until 4.5 to 5 mm carapace-width, we shall get an animal of the size of our ♀ 5. Accordingly, I would regard stage 3b of Atkins as being a very late stage 2. In actual fact the proportions of the abdomen and its segments are quite close to those of stage 2 (maximum breadth of abdomen 68.1 per cent. of carapace-breadth in stage 3b, as against 62.3 per cent. in stage 2, 34.7 per cent. in stage 1 (♀ 2) and 95.7 per cent. in stage 3a). During the period between stage 1 and stage 3 (Atkins stage 3a), the relative growth of abdomen-breadth must be very high (k in the heterogony formula over 2); after this, judging by the three points available, a definite but moderate heterogony with k about 1.3 appears to supervene. Similar variations in the time of onset of heterogony have been found with the male chela of *Maia* (Huxley, 1927), the female abdomen and the male chela of *Inachus* (Shaw), and is doubtless not uncommon. Atkins herself states (p. 477) that one stage 1 female was found with more approximation to the female form of abdomen than usual: and also that crabs have been found "with the abdomen reaching as far forward as in stage 4, but as wide as in stage 5, and *vice versa*." Clearly a marked heterogony in breadth and a minor heterogony in length both occur; these are (a) continuous processes, broken up into steps by the accidents of moulting; (b) the time of the onset of one is probably the same as that for the other, but this may vary relative to the size of the animal; and (c) the quantitative relation between length-heterogony and breadth-heterogony

may vary from individual to individual (just as it does in man, giving asthenic and pyknic types.)

The only male figured is not far from the smaller female of stage 1 in its abdominal proportions, but all save one of its segments are inferior in proportionate breadth (per cent. of carapace); and while its basal segments are inferior in proportionate length, its distal segments (5-7) are superior.

It is clear that a detailed study of the relative abdomen growth of this species, using really accurate measurements and reasonable numbers, would be of interest.

PART 2. NEGATIVE GROWTH-CENTERS IN THE WALKING LEGS OF THE RACING CRAB *Ocypoda ceratophthalma*

In a recent paper, Cott (1929) describes the biology of this typical semi-land crab. In it he gives a few measurements, showing that it has relatively very long legs in relation to its running habits, and that its young are precocial and, accordingly, like those of the ungulates, have relatively longer legs than the adults.

Measurements made by Hammond (See Part 3 of this paper) on the growth of the limb-segments in sheep show that during the marked relative decrease (negative heterogony) of these organs during the early years of life, there is a *reversed* growth-gradient, with center of least growth in the carpus and tarsus, while the proximal segments (humerus and femur) are growing more rapidly than any other parts of the limb. Thus, when as in crustacean chelae there is marked positive heterogony, the center of maximum growth is sub-terminal (propus); and when as in these Sheep limbs there is negative heterogony, the center of minimum growth is also sub-terminal. This suggested that the growth-gradients of positively and negatively heterogonic organs would be similar in form, but with sign reversed throughout, and prompted me to search for further examples. *Ocypoda* was such an example, and I wrote to Mr. Cott asking if he had measurements of the separate segments of the

walking limbs, in addition to the total lengths which alone are given in the paper. He kindly took measurements on 7 specimens, which were all he had available, and put them at my disposal.

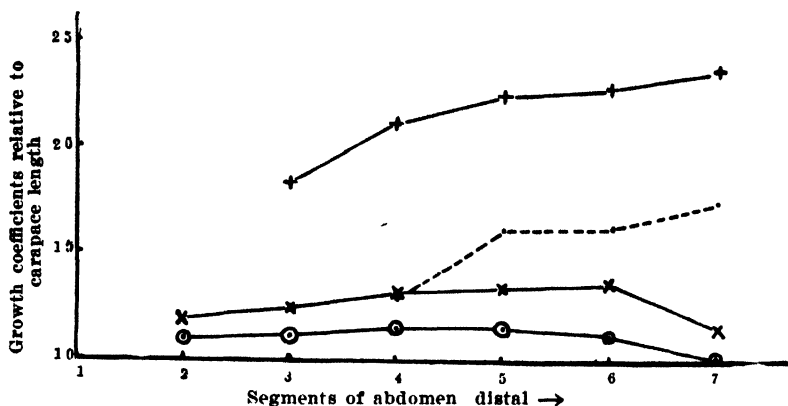


FIG. 1. Growth-gradients for abdominal breadth in ♂ *Telmessus*, ○; ♀ *Telmessus*, ×; ♀ *Pinnotheres*, +; and for abdominal length, ♀ *Pinnotheres*, dotted line.

Now in *Eupagurus prideauxi*, the only crustacean on which I could find detailed measurements of the separate segments of the pereopods, (Bush 1930), these limbs show distinct but slight positive heterogony; and in them there is a moderate double gradient with growth-center in the merus.

After plotting Cott's figures in various ways, I came to the conclusion that the best way to treat them for my purpose was to take the mean for the two smallest crabs, of 3.7 and 4.0 mm carapace length, both immature, and for the two largest crabs, of 28.5 and 30 mm carapace length, one ♀ and the other ♂ (sex appears to make little difference to the relative size of the pereopods in this species), and to see what the increase of the various segments was for this increase of carapace length. The results are tabulated below. The growth coefficients (k) relative to carapace length have been calculated as in Pt. 1 for two of the limbs.

TABLE IV
Ocypoda WALKING LEGS
 (Measurements in mm)

	Small	Large	Ratio Large: small	Growth coefficients (k)
Mean carapace length	3.85	29.25	7.60	
Pereiopod 1, total 1	13.45	87.50	6.48	0.93
Segments I-III	1.85	14.75	7.97	1.03
“ IV	4.35	25.75	5.92	0.88
“ V	1.60	10.75	6.73	0.94
“ VI	2.80	19.25	6.88	0.95
“ VII	2.85	17.25	6.06	0.89
Pereiopod 2, total 1	15.15	96.25	6.35	
Segments I-III	1.95	16.25	8.34	
“ IV	5.00	29.50	5.90	
“ V	1.70	11.00	6.48	
“ VI	3.45	21.75	6.31	
“ VII	3.05	17.75	5.83	
Pereiopod 3, total 1	14.45	93.00	6.50	
Segments I-III	1.80	15.00	8.34	
“ IV	4.80	28.75	5.99	
“ V	1.70	11.50	6.77	
“ VI	3.40	21.25	6.25	
“ VII	2.75	16.50	6.00	
Pereiopod 4, total 1	11.15	70.00	6.28	0.91
Segments I-III	1.60	11.75	7.35	0.98
“ IV	3.85	21.50	5.58	0.85
“ V	1.35	9.75	7.22	0.97
“ VI	2.25	15.00	6.67	0.93
“ VII	2.10	12.00	5.72	0.86

For the cheliped different measures must be taken, as it had been lost in the large ♂, and as this organ may be expected to be less well-developed in the ♀. I therefore take its increase from the same two small specimens to the largest complete ♂, with carapace-length 16 mm. We then have the result shown in Table V.

These results are thus given graphically in Fig. 2. Too much reliance can not be placed upon the precise values of k, but the fundamental similarity of the gradients for the walking legs is clearly shown and their fundamental difference from that for the chela. The cheliped behaves entirely differently from the other limbs. It is practically isogonic with the carapace; and has its growth-center in the merus, like the pereiopods of

TABLE V
Ocypoda ♂ CHELA

	Small	Medium	Ratio Medium: small	Growth coefficients (k)
Mean carapace length	3.85	16.0	4.16	
Cheliped, total 1	9.35	39.0	4.17	1.00
Segments I-III	1.70	7.0	4.11	0.99
“ IV	2.20	10.0	4.55	1.06
“ V	1.40	6.0	4.28	1.02
“ VI (to tip of ‘finger’)	4.05	16.0	3.96	} 0.96
“ VII	not separately measured			

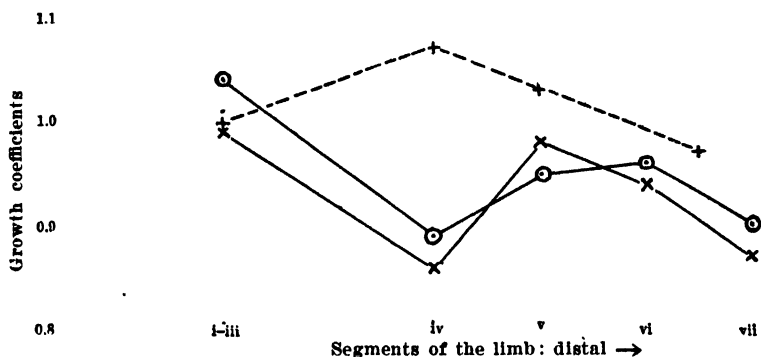


FIG. 2. Growth-gradients of the 1st (○) and 4th (×) pereopods and the cheliped (dotted line) of *Ocypoda ceratophthalma*. (The last point for the cheliped is placed between segments VI and VII, since the measurement was made to the tip of the propus finger, which includes the length of VII.).

Eupagurus, and like the cheliped of that animal so long as it is but slightly positively heterogonic.

The pereopods, on the other hand, show definite though not large negative heterogony: and their "negative growth-center" (center of minimum growth) is invariably in the same segment which in the slightly positively heterogonic pereopods of *Eupagurus* is the positive growth-center, the merus. The idea with which we started is thus in this respect confirmed.

There is not, however, a regular reverse growth-gradient from this point up in both directions; the ratios, after rising from the carpus, sink again for the propus and

dactylus (except in ppd 1, where they rise slightly for the propus).

This may have something to do with the fact commented on by Cott (p. 763) that in *Ocypoda* not only are the walking legs relatively long, in comparison with less active forms, throughout life, in spite of their negative heterogony relative to the body, but that this length has been achieved largely by a lengthening of the propus and dactylus.

The total length of the second walking leg in a specimen of the slow-moving crab *Sesarma meinerti* of carapace breadth 41 mm was 748 mm, as against 100 mm for that of an *Ocypoda* of the same carapace length. The ratio of the *Ocypoda* to the *Sesarma* leg is 1.34; but the ratio of the lengths of the various segments is as follows (Cott's Table I): I-III, 1.22; IV, 1.33; V, 1.05; VI, 1.57; VII, 1.45. It would appear that to achieve the excessive leg-length, a shift in the distribution of growth-intensity, presumably in very early life, has taken place, giving a second growth-center in the propus during the period of very rapid growth. The same phenomenon is witnessed in the right male cheliped of *Eupagurus*, when it changes from slight to marked positive heterogony, (Bush, *l.c.*).

In addition, the dactylus is extremely slender and tapering; thus a purely linear measurement gives too high a value for its total growth-intensity in a way which does not apply to the more or less quadrangular oblongs of propus and merus; a weight-measurement is really required.

The only other point which needs noting is the curious fact that in pereopods 1-3 the relative length-growth of segments I to III is actually higher than that of the body, although the limb as a whole shows negative heterogony. This phenomenon again should be investigated on the basis of weight-measurement.

It is clear from these preliminary results that the detailed investigation of two not too-distantly related

forms, one showing positive, the other negative heterogony of a corresponding part, would be of considerable value.

PART 3. NEGATIVE GROWTH-GRADIENTS IN THE LIMBS OF SHEEP

Hammond (1927 and 1929) has published certain interesting facts concerning the growth of the separate parts of the skeleton in sheep, indicating definitely the existence of growth-gradients in the limbs; but he has only given the relative weights (relative to the cannon-bone) not the absolute weights of the different parts.

He has kindly put his original data for the Suffolk breed of sheep at my disposal, and the following pages represent an analysis of these.

Measurements exist for 15 specimens, from new-born to adult, some males, some females, some castrated males. I have concerned myself only with the data for the different parts of the limb-skeleton (including limb-

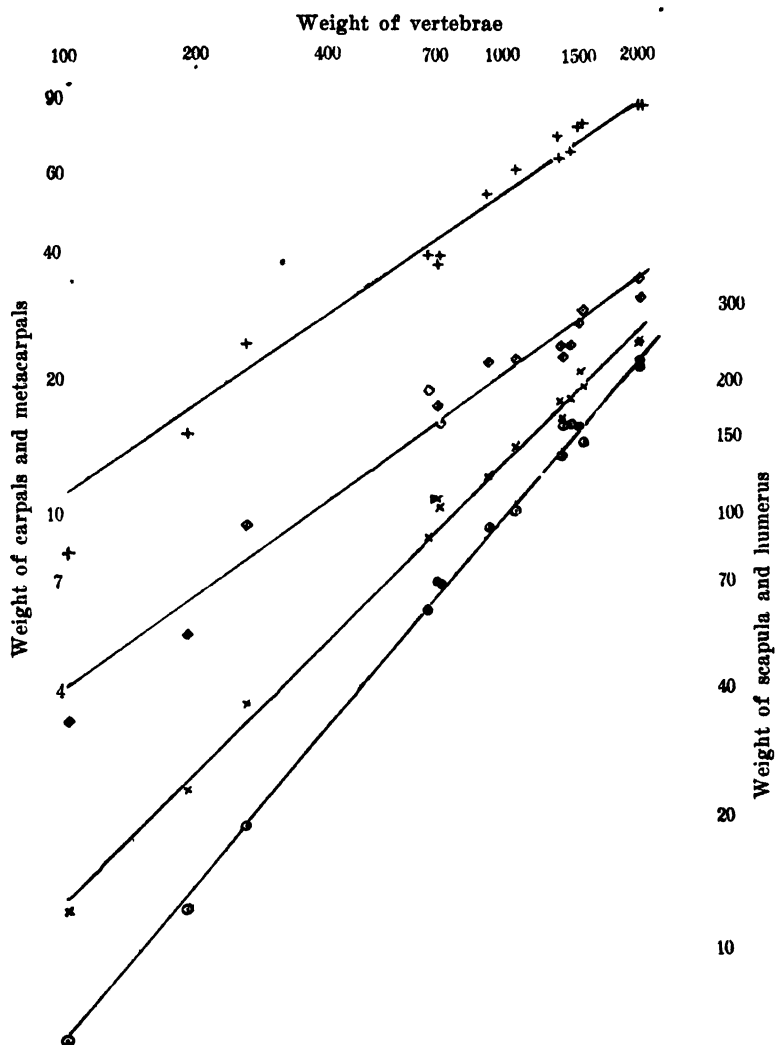
TABLE VI

WEIGHTS (GRAMS) OF SELECTED PARTS OF THE SKELETON IN SPECIMENS OF SUFFOLK SHEEP, FROM HAMMOND'S DATA. THE ROMAN FIGURES DENOTE THE REFERENCE NUMBERS OF HAMMOND'S SPECIMENS; WHERE MORE THAN ONE SUCH NUMBER APPEARS, MEANS HAVE BEEN TAKEN. k_1 , k_2 AND k_3 DENOTE THE VALUES OF THE GROWTH-COEFFICIENTS OF THE SEVERAL PARTS, RELATIVE TO THE VERTEBRAL COLUMN, FOR THE THREE SELECTED PHASES OF SIZE-INCREASE

	II	IV	k_1 , V+IX + X		k_2 , XV+XX	k_3
vertebrae	100.1	256.4		690.0	2075.5	
scapula	6.2	19.2	1.20	66.2	1.25	1.09
humerus	12.3	36.6	1.16	99.2	1.01	0.82
radius + ulna	12.08	35.7	1.15	78.7	0.80	0.84
carpals	3.35	9.4	1.10	18.2	0.67	0.53
metacarpals	8.15	24.5	1.17	44.5	0.60	0.59
pelvis	17.6	33.0	0.67	185.0	1.74	0.94
femur	16.0	49.6	1.20	126.5	0.95	0.57
tibio-fibula	15.18	45.8	1.17	105.3	0.84	0.62
tarsals	8.88	20.8	0.90	41.7	0.70	0.38
metatarsals	7.65	23.3	1.18	43.2	0.62	0.49

girdles) and as standard to plot these against I have taken his figures for the weight of the whole vertebral column: See Table VI.

Fig. 3 represents a double-logarithmic plot of the different parts of the fore-limb (omitting the figures for the



* FIG. 3. Double logarithmic plot of weights of scapula ○, humerus ×, carpals ◇, and metacarpals +, against weight of vertebrae in 15 Suffolk sheep (Hammond's data). The lines for each set of points are only inserted as visual aids.

radius-plus-ulna, as these overlapped those for the humerus and the scapula in a confusing way). It will be seen that there is, as one would expect, considerable variation, but that the points tend to be distributed along straight lines, indicating the existence of constant differential growth-ratios for the parts concerned. The first three points, however, especially as regards the carpals and metacarpals, are rather aberrant. This may perhaps be accounted for by the fact that these three animals, though of very different absolute size, were all of the same age (new-born); the smallest was one of triplets, the largest was a single birth, the intermediate specimen one of twins.

The corresponding figures for the parts of the hind-limb skeleton are similar, but a little more irregular, notably for the three first animals.

In order to obtain a graphic presentation of the growth gradients in the limbs, I have calculated the value of k , the growth-coefficient of the part, in the heterogony formula $y = bx^k$, from the formula

$$k = \frac{\log y' - \log y}{\log x' - \log x}, \text{ where } y' \text{ and } x' \text{ are the values for the}$$

part at a higher absolute size, y and x those at a lower value. I have calculated the coefficients for three portions of the size range: (a) from the smallest to the largest of the three new-born specimens; (b) from the largest of the three new-born specimens to the mean value for the three specimens with vertebral column weight close to 700; (c) from this latter point to the mean for the two largest specimens.

The relevant figures are given in Table VI.

Graphic representation of the results for all three phases in the fore-limb, and of those for the two last periods in the hind limb, are given in Fig. 4.

If we first look at the graph for the central period, we find in both extremities a remarkably smooth growth-gradient, with center of minimum growth in the sub-terminal region (unfortunately Hammond has not de-

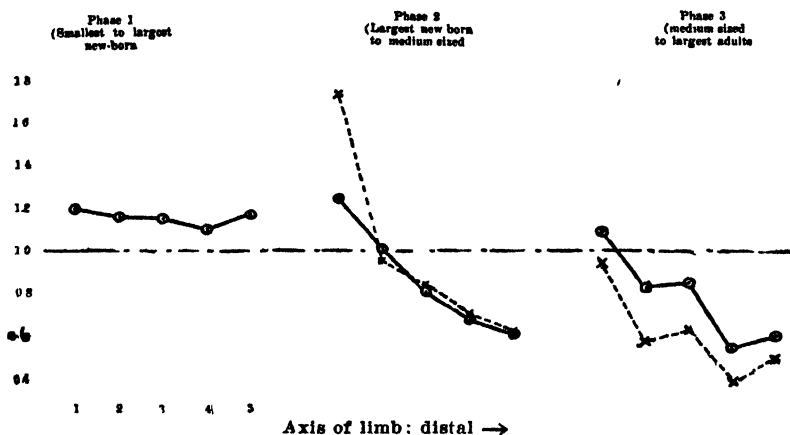


FIG. 4. Growth-gradients of sheep limbs in 3 size-phases. The distal end of the limb is to the right. Forelimb, solid line; hindlimb, dotted line. In each phase, 1 = limb-girdle; 2 = humerus; 3 = tibio-fibula; 4 = tarsals or carpals; 5 = metatarsals or metacarpals.

terminated the weights of the phalanges) and a steady increase of growth-ratio as we pass towards the body and in to the girdle.

The same type of gradient is seen in the last phase, but is somewhat irregular for both limbs. Such irregularities, however, are only to be expected with so few specimens. In general, the results confirm the views previously set forth (Huxley 1927), *viz.*, that when an organ is growing at a markedly different rate from the body as a whole, it will be found to show a growth-gradient. When it is growing faster than the body, we shall expect to find the growth-center or point of maximum growth at or near the distal end of the limb: this is realized in the chelae of Crustacea, and the abdomens of female Brachyura. Here, however, the limb exhibits negative heterogony: and we find a reversal of the sign of the growth-gradient, with center of minimum growth near the distal end.

It will be seen that the pelvis is growing faster than the vertebral column as a whole in both cases. This, Hammond's data further show, is associated with a high growth-rate of the lumbo-sacral region of the backbone as against the other regions.

The relative growth-rate of the whole limb sinks between the second and third phase; in both cases the limb as a whole is growing slower than the backbone. In the first phase, the growth-gradient is quite different, approximating to a straight line. Furthermore, the growth-coefficient of the limb as a whole, and of all its parts, is higher than that of the vertebral column. We may suppose that the small size of the smallest individual, due to its being one of triplets, has prevented it completing the previous growth-phase which is usually finished during intra-uterine life. During this, the limbs must clearly be exhibiting positive heterogony, thus causing the young animal to be born with disproportionately long limbs. Further, the growth of the scapula in this phase is lower than in the next phase. The growth-gradient for the hind limb in this phase (not reproduced in the figure) is more irregular, but in general similar; the value for the growth-ratio of the pelvis is very low.

We may therefore suppose that the sequence of events is somewhat as follows:

- (1) Strong positive heterogony of the limbs, which then presumably possess a positive growth-gradient with growth-center near their distal ends.
- (2) Increase of pelvis growth, and decrease of terminal limb growth, resulting in a flattening of the gradient (stage represented in our "first phase.")
- (3) Continued increase of pelvic (and presumably lumbo-sacral) growth, onset of marked negative heterogony of the limb. Result, a steep growth-gradient with negative growth center distally (our "second phase").
- (4) Continued negative heterogony of the limb, reduction of pelvic growth (our "third phase").

Mr. Hammond points out that different bones grow in different ways. Most bones grow by a combination of epiphyseal growth and general thickening, but the carpals and tarsals have no epiphyses and must rely solely on the second method. Then in the radius and ulna the epiphyseal growth continues longer than in the humerus. It is possible that minor discrepancies in the growth-gradients could be explained by reference to such facts.

In conclusion, I would like to thank Mr. Hammond for kindly allowing me to utilize his data, which at the moment of writing are unpublished, although they are shortly to appear in a book.

PART 4. DISTRIBUTION OF GROWTH-ACTIVITY IN THE METAMORPHOSING HERRING

In a recent paper Ford (1930) has discussed the changes in proportion in the herring during its transformation from the eel-shaped larva of rather less than 35 mm to the adolescent fishlet, with more or less the adult form, of 55 mm or more in total length. He finds that the head increases rapidly in relative size throughout the process, as does the ventral region between anus and end of trunk (root of tail) and the dorsal region from the first ray of the dorsal fin (D_1) and the end of the trunk.

The ventral distance from the pelvic fin to the anus, on the other hand, and the dorsal distance from hind end of head to D_1 decrease markedly in relative size; the ventral distance from hind end of head to pelvis decreases slightly in relative size. The increases and decreases are taken relative to body-length without tail. The tail stays of approximately the same proportion throughout, one seventh of total length. As a result of these complex changes, D_1 , the first dorsal fin-ray, moves forward relative to the vertebral column, the pelvic fins backward; whereas in the larva the pelvis are 6 vertebrae anterior to D_1 , in the metamorphosed fishlet they are 3 vertebrae posterior to it.

It occurred to me that it would be of interest to analyse the data from the point of view of relative growth-ratios, but the data presented in the paper did not permit of this analysis. However, Mr. Ford kindly put his original data at my disposal and discussed several difficulties with me.

It speedily became evident that only animals from samples of approximately the same date were comparable (see below). The following remarks accordingly

apply to 183 animals caught in the Tamar on 2/5/29 and 18/5/29 respectively. Relative head-length appears to be much the best criterion of metamorphic stage, and the animals were accordingly grouped into 12 classes from 10 per cent. to 21 per cent. head length, the means for the various regions of the body determined for each class, and plotted on double logarithmic paper against body-length. The results are shown in table VII and Fig. 5. On the whole, close approximations to straight lines are obtained, showing that the regions of the body are maintaining constant growth-ratios during metamorphosis:

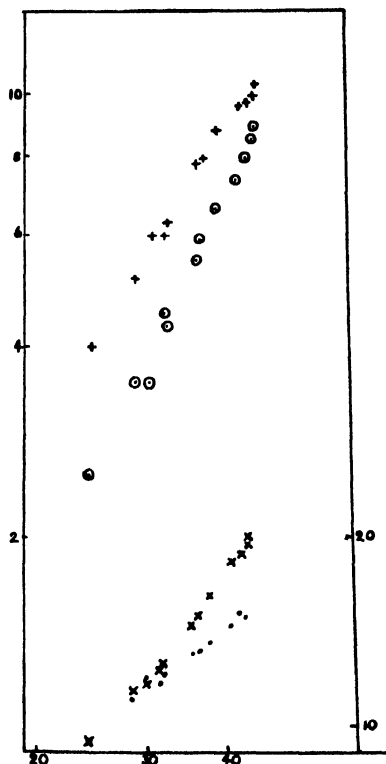


FIG. 5. Double logarithmic plot of the lengths of various regions of 182 metamorphosing herring grouped in 12 classes by relative head-length. Abcissa, total length without tail (A-E in Fig. 6a) $\odot$ head length. (A-B). $+$ anus to root of tail (H-E). $\times$ 1st dorsal fin-ray to root of tail (C-E). $\odot$ pelvis to anus (G-H). The scale on the left refers to the first two measurements, that on the right to the last two. Measurements in mm.

deviations, if present, are mainly in the extreme end-classes.

TABLE VII
MEASUREMENTS (MM.) OF DIFFERENT REGIONS OF THE BODY IN 182 METAMORPHOSING HERRINGS ARRANGED IN CLASSES BY RELATIVE HEAD-LENGTH

Class	Head l. % of A-E	M	Length to root of tail (A-E)	Head l. (A-B)	Pelvis to anus (G-H)	Anus to root tail (H-E)	1st dorsal to root tail (C-E)	Head to pelvis (B-G)	Head to 1st dorsal (B-C)
I....	10.0-10.0	2	24.0	2.5	8.0	4.0	9.5	9.5	12.0
II.....	11.0-11.9	7	29.9	3.5	8.5	6.0	11.7	11.9	14.7
III.....	12.0-12.9	12	28.2	3.5	8.6	5.1	11.3	11.0	13.4
IV....	13.0-13.9	16	31.6	4.3	8.9	6.3	12.7	12.1	14.6
V.....	14.0-14.9	19	31.1	4.5	8.9	6.0	12.3	11.7	14.3
VI.....	15.0-15.9	10	35.0	5.5	8.6	7.8	14.4	13.1	15.1
VII.....	16.0-16.9	18	35.6	5.9	8.6	7.9	15.0	13.2	14.7
VIII....	17.0-17.9	18	37.3	6.6	8.3	8.8	16.1	13.6	14.6
IX ..	18.0-18.9	29	40.1	7.4	8.4	9.7	18.3	14.6	14.4
X...	19.0-19.9	29	41.6	8.0	8.6	9.8	18.8	15.2	14.4
XI ..	20.0-20.9	18	42.4	8.6	8.7	10.1	19.5	15.0	14.3
XII	21.0-21.9	4	42.8	9.0	8.3	10.5	20.0	15.0	13.8

The letters refer to Fig. 4. A-B is head length; G-H, pelvis to anus; H-E, anus to root of tail; C-E, first dorsal to root of tail; B-G, end of head to pelvis; B-C, end of head to first dorsal.

As results we can say that the measured regions of the body fall into three groups:

(A) Those with positive heterogony relative to body-length.

(1) Head-length (A-B); growth ratio about 2.25.

(2) Ventral distance from anus to end of trunk (H-E); growth-ratio about 1.65.

(3) Dorsal distance from 1st dorsal fin ray (D1) to end of trunk (C-E); growth-ratio about 1.42.

(B) Those which show absolute increase of size but negative heterogony.

(4) The ventral distance from hind margin of head to pelvic fins (B-G); growth-ratio about 0.77.

(C) Those which show no absolute increase of size, but remain stationary or even regress.

(5) The dorsal distance from hind end of head to D1 (B-C).

(6) The ventral distance between pelvic end fins and anus (G-H).

Both of these, omitting the first size class, show *negative growth-ratios*, of about—0.05 in No. 5 and rather more in No. 6.

These results are shown graphically in Fig. 6a. In addition, by examining Ford's drawings it would ap-

pear (a) that the ventral surface of the head grows more than the dorsal, resulting in the shift of the mouth from ventral to dorsal of the anterior end; (b) that the region in front of the eye grows most rapidly of any in the head, and the eye itself next, while the region from eye to hind margin of head is growing only about as fast as the body as a whole; (c) that the relative length of the dorsal fin remains approximately constant, which means that it is the region posterior to this which is responsible for the positive heterogony of the dorsal distance D 1—end of trunk; (d) that in the region from anus to end of trunk, the major amount of heterogony is due to the growth of the anal fin region, whereas the region behind this remains almost constant relative to trunk length. In these points, however, Mr. Ford tells me that his drawings do not pretend to great accuracy, so that quantitative results can not be expected.

On the basis of this analysis, I have constructed Fig. 6, which gives a rough idea of how growth-intensity is distributed to various regions of the dorsal and ventral surface of the metamorphosing herring. We may call this the animal's growth-profile. The ordinates denote growth-ratios—the ratio of the length-growth of the part in question to that of the length-growth of the body as a whole, as denoted by the exponent k in the heterogony formula $[\text{part} = b. (\text{body})^k]$. Values over 1 indicate positive heterogony, below 1 negative heterogony. 0 indicates that the part is stationary in absolute length (I have not attempted to indicate absolute decrease in length).

It will be seen that the ventral surface shows higher growth-ratios than the upper. The shift of pelvic and dorsal fin *relative* to each other is caused by the presence of a ventral zone of growth anterior to the pelvics, of no-growth posterior to them, whereas the opposite is the case dorsally with regard to D 1. The anterior end of the head seems to be much the most rapidly growing region of the whole body, and more so on the ventral than on the dorsal side.

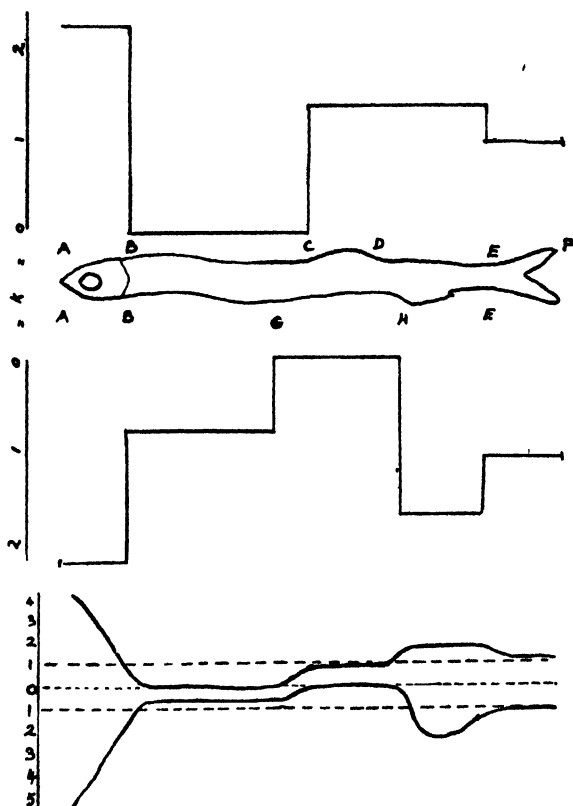


FIG. 6 (a). Outline drawing of young herring at onset of metamorphosis, from Ford (1930). Superimposed on it is its growth profile, constructed from the growth-coefficients for the various regions of the body, dorsal and ventral, as determined from the measurements in Table 6. (b) Growth-profile (on a smaller scale) of the same animal, constructed by taking into account drawings of different stages as well as measurements (see text).

The figure is of course only a first approximation to the truth. By making a detailed investigation of the length-changes of other regions, especially of the separate vertebrae, very interesting results could be obtained, and we might expect to obtain a series of growth-gradients instead of these regions of abruptly differing growth ratios.

It remains to be noted that in a sample of metamorphosing herrings taken on July 16, 1928, the proportions of the different regions were rather different. There

were some smallish animals with head-length taking up 23 and 24 per cent. of body-length. In these, head-length was right above the line determined for the 1929 samples. There were also a considerable number of very large animals, up to 90 mm body-length, with head-length only 19 or 20 per cent. In these, the head-length was well below the continuation of the 1929 line, and other proportions indicated that they were only about half as far metamorphosed as 1929 animals of about half their body-length. Presumably temperature and food can markedly alter the size and proportions at which metamorphosis is initiated, as is the case of course in, *e.g.*, Amphibian larvae. Here again an interesting field is open for further studies.

I must conclude this section with my best thanks to Mr. Ford for his kindness in putting his data and his experience at my disposal.

PART 5. THE NORMAL AND REGENERATING CHELA OF *Portunus sayi*

Zeleny (1908) gives interesting data on the normal growth and the regeneration of the chela of the Gulfweed crab, *Portunus sayi*. He gives the length of the propodite of the right chela and also the length of the carapace for a number of specimens from about 3 to about 15 mm carapace length. These results, extracted from his tables and tabulated by me according to body-length classes, are given in Tables VII and VIII, and are plotted on a double logarithmic grid in Fig. 7. It is seen that they give an excellent approximation to a straight line, from the slope of which we can calculate the growth-coefficient (k) of the chelar propus length to be about 1.15. There is further ~~an~~ indication of a double sinuosity in the curve, the points up to carapace-length about 5.5 showing a slightly higher growth-ratio, those from 5.5 to 9.5 a slightly lower one. This may perhaps be associated with breeding and non-breeding seasons, which we know to affect the growth of the male chela of *Inachus* (G. W. Smith 1905). In passing, the last column of Table VII

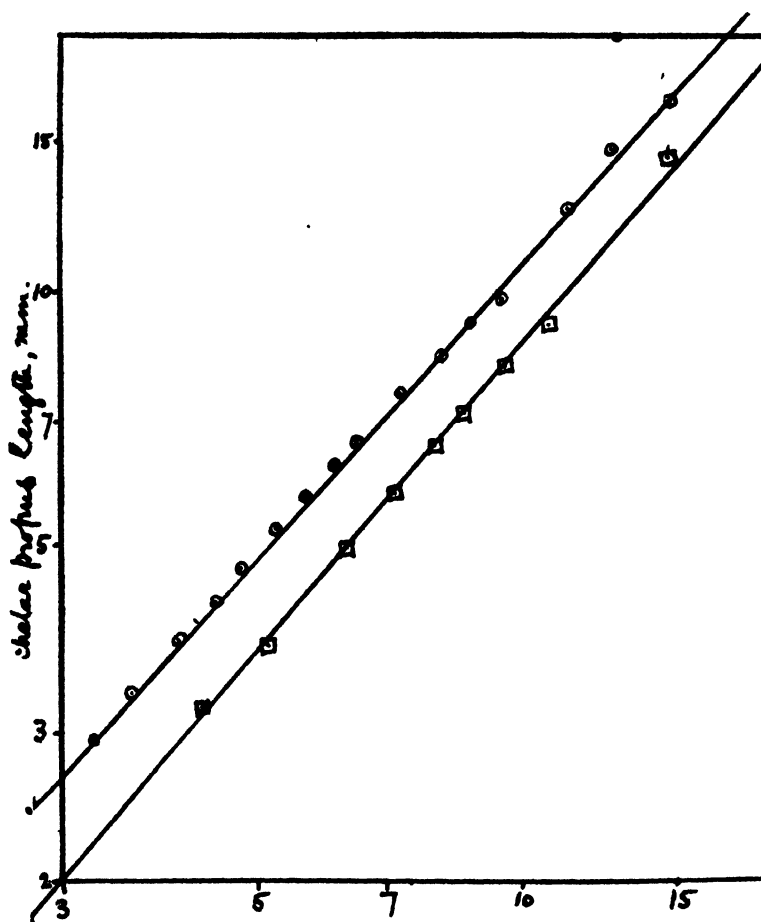


FIG. 7. Double logarithmic plot of normal ($\circ$) and regenerating ($\square$) right chelar propus of *Portunus sayi* against carapace-length (data of Zeleny 1908).

shows that there is no significant change in the ratio between R/L than with growth.

Zeleny also undertook a large number of experiments on regeneration. These are very valuable for our purpose, as all the results are comparable in every respect except size of animal at operation. After first ascertaining that the chief essential factor regulating amount of regeneration was moult-period, irrespective of the absolute amount of time occupied, he proceeded to do all his operations immediately after one moult, and all his mea-

surements of amount of regeneration immediately after the next.

His data enable us to plot the amount of chela (propus length) regenerated during one instar against body-size (carapace-length). The points thus determined also fall onto a straight line on the double logarithmic grid. As regeneration is never complete after a single moult, this line is of course below that for the normal claws. In addition, however, it shows a slightly but distinctly different slope, with $k \approx$ about 1.20. Thus the amount of chela regenerated after total amputation during a single instar stands in a definite fractional relation to the normal size of the claw for the body-size at the moult ending that instar; but the fraction increases slightly with absolute size of the animal. In other words, large animals regenerate a slightly greater proportion of their chelae than do small ones. Assuming the slope of the lines in the figure to be correct, the smallest regenerating crabs regenerate less than 78 per cent., the largest ones over 82 per cent. of the normal chela for that body-size. The difference is not large, and experiments should be conducted on a species with a larger absolute size-range to see whether it is really significant. If established, it would appear to be surprising, but might be accounted for according to the well-known principle that the rate of regeneration increases with the amount removed.

Leaving on one side the question of this possible small alteration in the regenerated fraction with change of absolute size, we see the strict quantitative relationship between the process of normal and regenerative growth, which Przibram has repeatedly stressed.

Zeleny himself stresses the facts (a) that relative chela-size increases with absolute body-size and (b) that "the proportion between the amounts of regeneration of a chela and the length of the chela in uninjured individuals of the same size is a constant uninfluenced by the size of the animal," but I have felt this note to be useful in pointing out that both normal and regenerating chelae conform in their growth to the laws of constant coeffi-

TABLE VII
SIZE OF PROPUS OF CHELAE IN UNOPERATED *Portunus sayi*, ARRANGED BY
CARAPACE-LENGTH CLASSES. FROM TABLES OF ZELENY (1908)

Class	No. of specs.	Mean length (mm) of			Ratio R/L chelar propus, per cent.
		carapace	L. chelar propus	R. chelar propus	
1	11	3.25	2.97	2.95	99.4
2	15	3.60	3.28	3.35	102.0
3	15	4.06	3.86	3.88	100.4
4	16	4.47	4.23	4.29	101.5
5	12	4.79	4.59	4.67	101.7
6	11	5.24	5.21	5.22	100.2
7	10	5.65	5.71	5.79	101.1
8	16	6.04	6.11	6.195	101.1
9	11	6.47	6.43	6.56	102.1
10	10	7.21	7.45	7.58	101.7
11	6	8.00	8.28	8.35	100.9
12	3	8.63	9.00	9.10	101.1
13	4	9.40	9.675	9.775	101.0
14	3	11.13	12.40	12.53	101.0
15	2	12.55	14.40	14.55	101.0
16	2	14.70	16.55	16.90	102.1

TABLE VIII
LENGTH OF PROPUS OF RIGHT CHELA REGENERATED DURING ONE INSTAR IN
Portunus sayi, ARRANGED BY CARAPACE-LENGTH CLASSES. FROM TABLE
10 OF ZELENY (1908)

Class	No. of specs.	Mean length (mm) of	
		Carapace	Regenerated chelar propus
1	5	3.98	3.06
2	11	4.45	3.28
3	12	4.83	3.71
4	9	5.50	3.90
5	6	6.08	4.90
6	6	6.43	4.92
7	12	7.10	5.72
8	6	7.87	6.50
9	10	8.52	7.18
10	6	9.50	8.12
11	7	10.66	9.09
12	1	14.50	14.40

cient of growth-partition found for other organisms, and, further, that the second of Zeleny's conclusions cited is not strictly true, as the coefficient of growth-partition is slightly higher for regenerating than for normal chelae.

SUMMARY

A number of numerical data found in the literature have been analysed in relation to the idea of differential growth-ratios and growth-gradients. The following chief results have been obtained.

(A) *Crustacean abdomens*

(1) The female abdomen of *Pinnotheres* (data of Atkins) has, both for breadth and length, a steep growth-gradient with growth-center in the terminal segment. The growth-gradient for length is at a lower absolute level than that for breadth, but is steeper. The size at which this rapid differential growth begins appears to vary by at least one instar.

(2) The female abdomen of *Telmessus* (data of Sasaki) has a much flatter growth-gradient (only determined for breadth) with growth-center in the sub-terminal (6th) segment. The corresponding growth-gradient for the male abdomen is still flatter, with growth-center between the 4th and 5th segments.

(B) *Negatively heterogonic Crustacean limbs*

The Racing Crab *Ocypoda* (data of Cott) shows a negative heterogony of its walking limbs, whereas its chela is isogonic. In positively heterogonic Crustacean walking legs so far investigated, the center of maximum growth is in the merus. Here, the merus is the center of *minimum* growth. The growth-gradient for all the walking legs is of similar, rather complex, form, quite different from that for the chela, which resembles that for the immature chela of *Eupagurus*.

(C) *Negative growth-gradients in the limbs of sheep*

This association between negative heterogony and a center of minimum growth is also seen in the limbs of sheep (data of Hammond). Here, however, the growth-gradient (which includes the limb-girdles) is of simpler type. The form of the gradient alters markedly about the time of birth.

(D) *Growth-profile in young herring*

During metamorphosis, the herring (data of Ford) shows radical differences in the growth-intensity of dif-

ferent regions. Dorsal and ventral parts of the same region of the body may show different growth-coefficients.

Quantitative evaluation of these has permitted the construction of an approximate "growth-profile" for the whole organism.

(E) Analysis of Zeleny's data shows that the right chelar propus length of *Portunus sayi* has a nearly constant differential growth-ratio (coefficient of growth-partition) of about 1.15 relative to carapace-length, and that the coefficient of growth-partition for regenerating chelar propus after 1 moult-period is slightly higher, viz., about 1.20. Thus larger chelae regenerate a slightly greater proportion of propus-length during one moult period than do smaller chelae.

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INHERITANCE IN *NICOTIANA TABACUM*

XI. THE FLUTED ASSEMBLAGE

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THE studies herein reported in outline deal with a series of derivative forms of *Nicotiana tabacum* var. *purpurea* (U.C.B.G. 25/06, Setchell, 1912) which have arisen spontaneously under circumstances about to be described. Completion of the investigations will require some further years; but it seems justifiable to present a preliminary account of them at this time, in advance of the presentation of the details, in order that the relationship of the various forms to one another may be set forth in an intelligible fashion.

N. tabacum var. *purpurea* has been chosen arbitrarily as the base of reference in our experiments with this species. Maintained by self-fertilization since 1906, it has no doubt long since become completely homozygous, if this system of propagation can achieve such a result. Its high degree of uniformity makes it possible to detect and study minor departures from type which otherwise might pass unnoticed. Despite its prevailing constancy, it does produce occasionally variants, which, insofar as we have studied them, appear to be due mainly to irregularities in distribution of chromosomes. They probably arise more often as a consequence of non-conjunction than of non-disjunction; at any rate PMC figures at IM sometimes exhibit $23_{II} + 2_I$ chromosomes instead of the normal 24_{II} . One of the most frequently detected of the variants is the monosomic, fluted; and the present paper is confined to an account of new derivative forms which have appeared during our studies of it. Judging from these investigations, variation in chromosome number may be the starting-point for a variety of other kinds of genetic modification.

Fluted: Studies of fluted have already been reported in some detail (Clausen and Goodspeed, 1926). Phenotypically fluted is characterized by numerous minor departures from the normal type; shorter, more intensely colored flowers with a peculiarly fluted limb, a more compact inflorescence, slightly telescoped growth, somewhat smaller and rounder leaves more erectly borne on the stem, and other less pronounced differences, constituting together a readily recognizable complex of characters. PMC figures at IM uniformly exhibit $23_{II} + 1_I$ chromosomes; the single being a large chromosome, characteristically somewhat constricted in the middle. This single chromosome we shall call the F-chromosome; fluted, then, is haplo-F. Fluted ♀ x normal ♂ produces about 40 per cent. normal and 60 per cent. fluted offspring; but the reciprocal cross gives about 98 per cent. normal and only 2 per cent. fluted offspring. Evidently 23-chromosome gametes are present in the female series somewhat in excess of the 50 per cent. expectation, probably as a consequence of lagging of the univalent during meiosis; and 23-chromosome pollen grains are at a marked disadvantage in competition with normal 24-chromosome pollen grains. Fluted is ordinarily maintained by crosses of fluted ♀ x normal ♂ in order to insure genetic identity of the two types; and in our experience, under this system of propagation, the offspring has always consisted exclusively of normal and fluted individuals.

Fluted x self: When fluted is selfed the progeny usually consists of normal and fluted plants in about the same proportions as from fluted ♀ x normal ♂. Such populations have been grown on a number of occasions with the object of obtaining the 23_{II} , nullo-F form corresponding to fluted; but it has never been secured and it is probably an inviable type. However, two new derivatives, coral and mammoth, have been obtained from such populations, in both instances as single variant individuals from which constant stocks have been established.

Coral, named from the distinctive coral or salmon pink color of its flowers as contrasted with the carmine of normal and fluted, is a striking type. With its semi-dwarf habit of growth and small, light green leaves, it exhibits as marked contrasting features to normal vegetatively as in flower color. There are two forms of it, normal coral and fluted coral, which exhibit phenotypic differences parallel to those between normal and fluted. PMC preparations of normal coral show 24_{II} chromosomes, apparently indistinguishable from the normal chromosomal garniture; and those of fluted coral have $23_{II} + 1_I$, the single being of the same type as that in unmodified fluted (Clausen, 1930).

Mammoth is apparently the same character as is represented in such commercial varieties as Maryland Mammoth. It is characterized by slow but long-continued growth, leading to the production of tall, unbranched plants with numerous leaves. As it approaches maturity it branches in candlelabrum fashion at the top; but with us it ordinarily does not bloom until very late in the season, if at all. Grown in the greenhouse from fall sowings during the winter, however, it blooms only slightly later than normal plants. Evidently our type has the same response to length of day noted by Garner and Allard (1920) for the commercial mammoth. The blossoms are practically identical with those of normal both in size and color. Like coral, mammoth may exist in the normal and fluted conditions with 24_{II} and $23_{II} + 1_I$ chromosomes, respectively.

Both of these types are recessive to normal, the heterozygote in each case exhibiting only a slight departure towards intermediacy. In both instances, moreover, the new form has arisen by modification of the F-chromosome, as is shown by the following results:

fluted ♀ × coral ♂ → F₁ normal + fluted coral

fluted ♀ × mammoth ♂ → F₁ normal + fluted mammoth

Crosses of coral x mammoth produce normal F_1 , indicating that the modifications involve different elements of the F-chromosomes. Without for the present committing ourselves to the hypothesis of factor mutation as responsible for their origin, we may assign the formula F-co F-co to coral and F-mm F-mm to mammoth.

Coral x normal: As we have just stated, coral x normal produces normal F_1 . The F_2 progenies in the main closely approach the expected ratio of 3 normal: 1 coral; but they also contain scattering representatives of other types of which the following appear regularly:

large lax	$24_{II} + 1_I$,
fluted	$23_{II} + 1_I$,
fluted coral	$23_{II} + 1_I$.

The large lax type has flowers considerably larger than normal, with a wider throat and somewhat lighter color; the plants are taller, leaves longer and narrower and with reduced auricles, internodes longer, and the growth is characteristically lax, so that the plants often have crooked stems. In general, the large lax form exhibits the opposite kind of departure from the normal type as compared with that seen in fluted. Cytological examination reveals the presence of an extra chromosome, hence large lax is trisomic. In general features it closely resembles the trisomic which we have previously described under the designation enlarged (Clausen and Goodspeed, 1924).

The other two types, fluted and fluted coral, are identical, phenotypically and cytologically, with the forms previously described under these names.

The explanation for the appearance of these exceptional individuals in F_2 appears clearly to be afforded by cytological examination of F_1 plants, PMC preparations of which frequently exhibit $23_{II} + 2_I$ chromosomes instead of 24_{II} . It would appear that non-conjunction of the F-chromosomes occurs frequently in normal plants heterozygous for coral, as a consequence of which some

nullo-F and diplo-F gametes are produced. These account for the production of fluted and fluted coral plants in F_2 and also for large lax if this type is triplo-F. If so, large lax must be genetically distinct from the trisomic enlarged which has been shown not to involve the F-chromosome (Clausen and Goodspeed, 1926).

Besides the variant types which have just been mentioned and which appear to be due to non-conjunction of the F-chromosomes, a number of others have appeared in some F_2 progenies. Of these, we consider here only one, a type called pale sterile, which has appeared in approximately one fourth of the individuals of some, but not all, F_2 progenies. Pale sterile is somewhat paler as to flower color than normal and differs from it slightly in some other respects. Its characteristic feature is its pronounced sterility; for it produces practically no functional pollen, and judging from the results of pale sterile ♀ x normal ♂ crosses, only about 10 per cent. of its ovules are functional. Examination of PMC figures of pale sterile reveals extensive failure of conjugation as a consequence of which the figures have the appearance of those of interspecific hybrids, except that there appears to be no regular number of bivalents and univalents. Evidently pale sterile is an asynaptic form comparable to the one which Beadle (1930) has described in maize. Pale sterile appears to segregate independently of coral, hence, it does not appear to be due to a modification of the F-chromosome. The type has also been observed to segregate in some coral populations, hence it has probably been introduced with the coral in these crosses. It has not, however, been observed in other lines of *N. tabacum*.

Mammoth x normal: F_1 mammoth x normal is normal and F_2 progenies consist approximately of 3 normal: 1 mammoth. Apparently, however, the same sort of irregularities arise as in the crosses of coral x normal; but the populations have not been so satisfactorily class-

ified on account of the failure of mammoth plants to blossom during the normal growing season. As with coral, occasional fluted and large lax plants have been observed in F_2 progenies. Inasmuch as the mammoth plants did not bloom, it could not be determined satisfactorily whether or not any of them were fluted; but some of them certainly exhibited the peculiar vegetative characters of that type.

Coral x mammoth: As stated above coral x mammoth gives normal F_1 . The cross was made for the express purpose of determining the linkage relations of coral and mammoth, inasmuch as the crosses with fluted had shown them both to be due to modification of the F-chromosome. In the absence of a coral mammoth stock, the customary backcross could not be made. As a substitute crosses were made of fluted ♀ x F_1 coral x mammoth ♂ which should produce fluted offspring of various classes corresponding to the types of gametes produced by F_1 coral x mammoth. The results were as follows:

114	normal
52	fluted coral
58	fluted mammoth
2	fluted
0	fluted coral mammoth

Only the fluted classes are of significance for the problem at issue; fluted coral and fluted mammoth representing the parental combinations and fluted and fluted coral mammoth the recombinations. The figures presented might be interpreted to indicate about two per cent. of crossing-over between coral and mammoth. However, it is to be noted that no fluted coral mammoth plants were obtained and the two fluted plants may have arisen from 23-chromosome pollen grains produced as a result of non-conjunction of the F-chromosomes in F_1 coral x mammoth. Further evidence in support of this interpretation was afforded by the presence of seven large lax plants of the type previously described, which are in-

cluded among the normal plants in the above tabulation. If these plants are triplo-F, as has been suggested above, they should have the constitution, F F-co F-mm; i.e., their three F-chromosomes should be different.

However, included among the fluted coral plants in the above tabulation were three plants which were normal coral; and, although the same difficulties were experienced in dealing with the mammoth plants as in other populations, a few of them appeared from the vegetative characters to be normal rather than fluted. Apparently equational non-disjunction must be responsible for their appearance; thus we reach the conclusion that non-conjunction or the unpaired condition of a chromosome may predispose to consequent equational non-disjunction. In view of this fact, it is of course possible for the large lax plants to possess a variety of constitutions, instead of the one stated above.

Fluted x coral: As was stated above, fluted ♀ x coral ♂ gives normal and fluted coral offspring in F₁. In addition to these expected offspring, however, four separate instances of origin of carmine-coral variegation have been noted. These variegated plants had a carmine continuous and a coral disperse phase, in which respect they exhibit the opposite condition from that shown by the classical instances of variegation in which the recessive is the continuous and the dominant the disperse phase. The four instances of variegation were all different phenotypically, both as respects the type of variegation and the vegetative features of the plant. The first instance, carmine-coral-I, had vegetative features almost identical with coral; but the flowers, although of the same size as those of coral, are carmine; flecked, flaked and striped with coral. Somatic reversion to self-coral occurs frequently; to self-carmine rarely, if at all.

The other three instances arose from a cross of fluted mammoth ♀ x fluted coral ♂ and have only been seen in single plants as yet. However, carmine-coral-II had

vegetative characters close to normal and a coarser type of variegation. Carmine-coral-III also had vegetative characters closely approaching those of normal plants and flowers nearly normal in size; but the coral flecks were usually sparse and small. Carmine-coral-IV had somewhat larger flowers than those of carmine-coral-I; but had a close resemblance to it in vegetative features and type of variegation.

In carmine-coral-I it has been shown (Clausen, 1930) that the variegation is associated with the presence of a small, fragmentary chromosome, which is interpreted, in view of the parentage and the phenotypic effects, as a fragment of the F-chromosome bearing the carmine flower color complex, represented by f-Co. In terms of this notation normal carmine-coral is F-co F-co f-Co and fluted carmine-coral, F-co f-Co. The variegation appears to depend on sporadic loss of the fragment during development.

Carmine-coral x coral: Carmine-coral-I has been studied genetically in some detail through employment of a variety of crosses of normal and fluted coral with normal and fluted carmine-coral and through selfing of the two latter types. These results will be reported fully in the next following paper in this series. In the main they show a low and erratic ratio of transmission of variegation, amounting on the average to about fourteen per cent. both in the female and male series of gametes; and not infrequently individual carmine-coral flowers fail completely to transmit variegation. Doubtless this last behavior depends upon early elimination of the fragment; but the results are not consistent with the characteristic features of particular flowers, owing to the cell layer relations involved in flower color production (Clausen and Goodspeed, 1923).

Of outstanding significance, however, is the occasional production of self-carmine plants in progenies from variegated parents. Seven separate instances of this phe-

nomenon are on record; its ratio of occurrence thus far is about three for every hundred variegated plants. Just how this reversion to self-carmine has been accomplished has not yet been determined, but one possibility may apparently be eliminated at the outset. Four of the recorded instances arose in crosses of fluted carmine-coral ♀ x coral ♂, and all four of them were fluted self-carmine plants. They could not have arisen by reattachment of the fragment to the F-chromosome for these plants received their only F-chromosome from their paternal parent and the carmine came in from the maternal side.

Pale sterile x normal: Progenies grown from pale sterile ♀ x normal ♂ are highly variable, but the plants, with occasional exceptions, do not present any very extreme departures from the normal type. Preliminary cytological examinations of individual plants in such a progeny disclosed a variation in chromosome number such as might be predicted from the irregular distribution of chromosomes observed in pale sterile. Accurate counts were extremely difficult to make; but apparently the offspring were all modified diploids; i.e., they had $24_{\pm}$ chromosomes plus and minus, or in other words the plants were trisomic for some and monosomic for other chromosomes. No triploids were detected among them, in which respect, as well as in the production of monosomics, the results differ somewhat from those described by Beadle (1930) for his asynaptic maize. Employment of pale sterile, therefore, represents an addition to the already numerous methods of obtaining plants with variant chromosome numbers in *N. tabacum*, but it appears to be a particularly excellent one, for apparently one may obtain the entire series of primary monosomic and trisomic types from it.

DISCUSSION

With the possible exception of pale sterile, the foregoing series of forms all appear to involve variation

either in the number of F-chromosomes or in their constitution, for which reason we call them the fluted assemblage. Those which arise by variation in the number of F-chromosomes require no further comment; but those which are due to some internal modification of the chromosome present some rather interesting problems. The mutational sequence in these instances was of the form, normal $\rightarrow$ haplo-F $\rightarrow$ haplo-modified-F; and in view of the fact that the modified forms arose from fluted $\times$ self but not from fluted $\times$ normal, they appear to be due to some special form of modification to which the chromosome is occasionally subject when in the univalent or unpaired condition.

In mammoth we deal with a character the origin of which has been reported independently by a large number of investigators, and which has been utilized extensively in commercial practice. In general, genetic investigations with it support the conclusion reached by Allard (1919) that it is a simple recessive to normal; and the origin of the character has often been cited as a typical example of factor mutation. But it is at least not the usual thing for factor mutations to disturb the conjugation of the chromosomes in which they are located; and as for the evidence from genetic studies, it may be dismissed as inadequate, for any genetic alteration which behaves as a unit and does not impair the vitality or viability of the gametes and zygotes will give results superficially identical with those of simple factor differences. Moreover, various puzzling phenomena have been reported in connection with previous studies of mammoth which indicate that the same type of irregularity in chromosome distribution detected in our experiments has also occurred in them. Thus both Lodewijks (1911) and Honing (1914) reported the occurrence of an intermediate giant type, probably triplo-F, which segregated for normal. It may be recalled also that Hayes and Beinhart (1914) found that a few mutant mammoths

appeared in the progeny of normal plants which were apparently not heterozygous for the character, and felt impelled on the basis of this fact to conclude that the mutation must have occurred after fertilization. If, as is almost certainly the case, undetected fluted plants were present in their plantings, the mutational sequence, normal $\rightarrow$ fluted $\rightarrow$ fluted mammoth, may have been in operation in their instances as in ours. At any rate this explanation avoids the obvious difficulty of their interpretation, since the presence of a single F-chromosome in fluted makes it possible for recessive mutations in this chromosome to manifest their occurrence immediately.

More cogent evidence is afforded from the study of coral and carmine-coral variegation. In carmine-coral plants a fragment is present which bears a factor complex capable of restoring the normal carmine color, but leaving the no less marked vegetative features of coral only slightly altered. When this complex becomes stable, as in reversionary self-carmine, a type is produced identical as to flower color with normal but exhibiting an only slightly more robust growth than coral. The fragment, therefore, has a portion of the genetic material necessary to restore coral to the normal condition, but not all; or to put the matter in another way, the coral flower color and the vegetative features characteristic of the type are due to alteration of different germinal elements. Coral, therefore, depends upon some genetic modification more extensive than simple factor mutation.

The occurrence of fragmentation, as evidenced in the origin of carmine-coral variegation, suggests that a similar phenomenon on a less extensive scale may have been responsible for the origin of coral and mammoth. PMC preparations of fluted not infrequently exhibit the univalent in IA stretched out from one chromosome group to the other, suggesting that it may be torn in pieces simply as a consequence of its univalent condition. In view of the fact that nullo-F gametes are functional,

there seems to be no reason why a gamete containing any part of the F-chromosome should not also be functional, and the question as to whether the process described would give rise to new genetic types simply hinges on the possibility that zygotes arising from such gametes may be viable. In effect, this process would give rise to deficiencies, and in general those which have been studied heretofore, mainly in *Drosophila*, have been lethal when homozygous. But *N. tabacum* is probably a polyploid species (Clausen, 1928) and under these circumstances it would appear probable that deficiencies would have a less serious effect than in species such as those of *Drosophila*. On the cytological side, however, both in coral and mammoth, if the alteration is of the nature of deficiency, it is insufficient in extent to be recognizable cytologically. Since the modified F-chromosome is present in the univalent condition in fluted coral and fluted mammoth, it may be identified with certainty in meiotic divisions. Comparisons of the univalent chromosomes of these types with that of fluted seem to indicate that they are identical morphologically.

As an alternative to simple deficiency, the possibility might be considered that they represent a modification of the type postulated by Belling and Blakeslee (1924) to account for those secondary trisomics of *Datura* which contain an extra chromosome modified genetically but unaltered in size. Their suggestion is equivalent to a deficiency of one half of the chromosome and a duplication of the other half, and they have advocated an hypothesis of reversed crossing-over to account for it. The hypothesis at least is inapplicable in the present instances, for the modification occurred in individuals which had only one F-chromosome. Possibly a similar condition may arise by fragmentation of a single chromosome, inasmuch as a loss of part of the chromosome and a duplication of the remaining portion through the medium of the equational split might occur simultaneously.

Whatever the precise nature of the chromosomal modification responsible for the production of coral and mammoth, the results afford a demonstration of the possibility of obtaining new and distinctive races of *N. tabacum* by substitution of modified chromosomes, genetically altered in some way other than by factor mutation, for normal ones. In most instances when genetic modification has gone beyond the limits of factor mutation, barring those in which the elements are all present but with an altered organization, no complete replacement of the normal chromosome has been possible. Thus the secondary modifications of *Datura* (Blakeslee, 1924, 1928) are apparently obtainable only in the trisomic condition in association with two unmodified chromosomes. It is, of course, probable that, as in the case of mammoth, some instances hitherto ascribed to factor mutation or to some other kind of alteration, actually belong in the same category.

The speltoid and related forms of wheat studied genetically by Nilsson-Ehle (1921, 1927) and Linhard, (1922, 1927) and cytologically by Winge (1924), Huskins (1928b) and others and the fatuoid series in oats (Huskins 1927, 1928a) present many features in common with the fluted assemblage; but the details are so complex and the relation between the cytological and genetical findings so dubious, that it seems of doubtful value to attempt a discussion of the various explanations which have been offered for them, particularly as the whole situation has recently been reviewed by Watkins (1930). It may, however, be possible that some of the 42-chromosome fatuoid and speltoid derivatives owe their origin to secondary genetic modification arising as a consequence of a previous monosomic or trisomic condition, rather than to the replacement of chromosomes of one set by corresponding chromosomes from another or to crossing-over between corresponding chromosomes of different sets. Possibly the phenomena here described occur only in polyploid

species, but in view of the great number of such species in plants, this restriction would not greatly reduce their importance.

SUMMARY

1. By loss of an F-chromosome, probably as a consequence of occasional non-conjunction, *N. tabacum* var. *purpurea* produces the monosomic, fluted.

2. When selfed, fluted has produced as further variants the distinctive recessive types, coral and mammoth, both of which have been established in pure lines.

3. From crosses with fluted, both coral and mammoth have been shown to be due to secondary modification of the F-chromosome.

4. Coral and mammoth, when crossed with normal or with each other, produce hybrids of the normal type, which, however, exhibit frequent non-conjunction of the F-chromosomes.

5. By reason of this non-conjunction, F_2 and other derivative populations from these crosses contain some haplo-F and triplo-F individuals in addition to the expected classes of offspring.

6. Hybrids of coral with mammoth apparently exhibit no crossing-over between the two modified F-chromosomes.

7. Some of the F_2 populations of coral $\times$ normal segregated for a recessive pale sterile type, which owes its sterility to extensive non-conjunction of the chromosomes in meiosis.

8. Crosses of pale sterile $\text{♀} \times$ normal ♂ produce a variable offspring consisting of numerous monosomic, trisomic and other more complex types of chromosomal variants.

9. Crosses of fluted $\text{♀} \times$ coral ♂ and fluted mammoth $\text{♀} \times$ coral ♂ have produced four instances of carmine-coral variegation, one of which has been shown to arise from fragmentation of the F-chromosome.

10. Carmine-coral variegated plants produce occasional reversionary self-carmine variants, which are identical in flower color but not in vegetative features with normal.

11. Evidence from carmine-coral variegation and reversionary self-carmine indicate that the vegetative features of coral and its distinctive flower color must be due to alteration of different components of the F-chromosome; hence coral must represent some modification of the F-chromosome more extensive than simple factor mutation.

12. Evidently the F-chromosome in the univalent condition is subject to some special forms of genetic modification which occur rarely, if at all, in normal material.

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STUDIES IN HUMAN INHERITANCE. V.¹

MULTIPLE ALLELOMORPHISM AS OPPOSED TO LINKAGE IN BLOOD GROUP HEREDITY

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THE inheritance of the human blood groups has been shown to be dependent upon a set of triple allelomorphs (Bernstein, 1925, Snyder, 1926, 1929, *et al.*). Based on this interpretation, laws relating to legal and clinical medicine, and to anthropology, have been formulated (Snyder, 1927, 1929, 1930). The evidence for the triple allelomorph hypothesis was considered adequate, and the question of the mode of inheritance of the blood groups was, I thought, settled. Former suggestions of hypotheses, involving, respectively, two pairs of independent factors and two pairs of completely linked factors, had been shown to be untenable.

In 1928, however, a short note appeared by K. H. Bauer, suggesting the assumption of two pairs of partially linked factors, instead of triple allelomorphs, as the basis for the inheritance of the blood groups. This hypothesis was said by its proposer to explain the exceptions which had been observed by various workers in the offspring of certain crosses. Bauer assumed 11 per cent. cross-over between the two linked factors.

This hypothesis, however, is genetically as untenable as the old hypothesis of independent assortment. In my monograph on the blood groups (Snyder, 1929), I mentioned briefly the linkage hypothesis, stating that it could not adequately explain the observed facts, and giving concise specific reasons for my statement.

In spite of this, however, in two text-books which have just appeared, Bauer's hypothesis of linked factors is accepted.

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Gates, in his "Heredity in Man," p. 196, after reviewing the blood group data, puts forward Bauer's new hypothesis, saying that it "... appears to resolve the difficulties in the two previous theories." He also states that on this basis "the results appear to compare favorably with expectation." Likewise Castle, in the fourth revised edition of his "Genetics and Eugenics," just off the press, says, p. 372, "... On the whole, the recorded observations accord best with the second interpretation, which regards A and B as mutations in different genes borne in the same chromosome, and so with only occasional crossing over between them."

The acceptance of the hypothesis of linked factors in two such widely read texts as those cited makes it necessary for me to refute specifically the arguments advanced in favor of this explanation of the heredity of the blood groups.

Bauer's reason for advancing the linked factor hypothesis was that on the basis of triple allelomorphs some exceptional results are obtained. Specifically, in crosses of O and AB, only children of groups A and B should be obtained, whereas occasional children of groups O and AB have been recorded from such matings. The hypothesis of linked factors purports to explain these.

There are two points to be developed in refuting this claim. First, it is not at all certain that exceptions really exist. Second, if some exceptions to the hypothesis of triple allelomorphs do exist they can not be explained by assuming linkage. A possible genetic explanation for these, if they really exist, will be mentioned below.

To consider these two points, then, one at a time, let us examine the recorded exceptions. On the basis of triple allelomorphs, the genotypes of the four blood groups are as follows:

Group O - OO
Group A - AA, AO
Group B - BB, BO
Group AB - AB

Thus, crosses of group O with AB should give only children of groups A and B, as follows:

$$\begin{array}{c} OO \times AB \\ AO, BO \end{array}$$

Actually, some children of groups O and AB have been recorded from such crosses. However, the vast majority of such exceptions were recorded in the early years of blood grouping (1910-1925), before the technique was standardized, and before anything was known of the triple allelomorph hypothesis. Since 1925 exceedingly few exceptional cases have been recorded. This is shown in Table I.

TABLE I
OFFSPRING OF CROSSES OF GROUPS O AND AB

	Numbers in group				Per cent. Exceptions
	O	A	B	AB	
(1) Before hypothesis of triple allelomorphs	31	94	61	26	36.7
(2) Since hypothesis of triple allelomorphs	14	405	400	7	
(3) With known errors removed from (2)	7	401	399	5	1.5

It may be seen from this table that previous to the triple allelomorph hypothesis there were 36.7 per cent. exceptions to the rule as based on triple allelomorphs. Since this hypothesis was put forward, however, only 1.5 per cent. exceptions have been recorded, although the total number examined is four times as large. The second line in Table I represents all the recorded exceptions, while the third line gives those which remain after removing the known errors from the second line. For example, of the 14 exceptional Group O children recorded since the triple allelomorph hypothesis, two were known to be illegitimate (Snyder, 1, Furnhata, 1) and four were wrongly included because of the mixing of two families of the same name (Klieve and Nagel). Of the

seven exceptional group AB children, one was known to be illegitimate (Thomsen), and one was again the case of the confusion of two families of the same name (Kliewe and Nagel). With these removed, the percentage of exceptions is only 1.5, and this in spite of the fact that several investigators have concentrated specifically on this type of mating with the express purpose of looking for exceptions. The small remaining per cent. may be explained on the basis of illegitimacy, mistakes in technique, adoptions, mixing of babies in hospitals, or on the basis of non-disjunction, as will be shown later.

My own studies, involving more than 500 families, more than 25 of which are of O with AB, do not give any exceptions to the hypothesis of triple allelomorphs, except one case, which was on investigation shown to be a case of illegitimacy (Snyder, 1929). Personally, I am convinced that illegitimacy and the other possibilities just mentioned will fully account for any exceptions recorded.

Matings of groups O and AB are hard to obtain, since group AB is so rare. However, by taking the blood groups of mothers and children in maternity hospitals the problem may be indirectly attacked. Twelve investigators have done this, recording a total of 371 mothers and 371 children. The results are shown in Table II.

TABLE II
OFFSPRING OF GROUP AB MOTHERS

Number of families	Number in group			
	O	A	B	AB
371	2	196	150	175

It will be seen that the exceptions in this case are the group O children, since group AB children are possible inasmuch as the fathers could be of any of the four groups. Illegitimacy can not explain these, but mistakes in technique, adoptions, mixing of babies, etc., could. Of the 12 investigators, some of whom studied as many as a hundred cases, only one found exceptions. This worker

studied only seven families altogether, and recorded two exceptions.

The exceptions to the law of triple allelomorphs, then, are certainly not very numerous. Assuming, however, that some actual exceptions do occur, would they be explained by assuming linkage of the two factors concerned? In glancing at the last line of Table I, it would seem offhand that the second and third columns might represent non-crossovers, and the first and fourth might represent crossovers. Bauer so interpreted these columns, although he used the total results, and did not separate them into those recorded before the triple allelomorph hypothesis and those recorded afterwards.

Castle and Gates both interpreted the four columns as crossovers and non-crossovers. All three of these workers, however, have apparently completely lost sight of the fact that in a random-mating population the coupling and repulsion phases are not conveniently separated, as they are in cages of experimental animals, but that both phases will occur, *and in equal proportions*, even assuming only a very small per cent. of crossing over. Time is the only requisite. This would mean that the four columns in Table I should be approximately equal on the basis of linkage. Complete equality would never occur because of the presence of some single and double homozygotes among the group AB individuals. This point may profitably be examined in detail.

Let us, for the sake of argument, assume that the blood groups are, as Bauer suggests, inherited as linked factors. Then the genotypes of the four groups would be as follows:

Group O	(ab)(ab)
Group A	(Ab)(Ab), (Ab)(ab)
Group B	(aB)(aB), (aB)(ab)
Group AB	(AB)(AB), (AB)(Ab), (AB)(aB), (AB)(ab), (Ab)(aB)

Bauer and those who accept the linkage hypothesis apparently consider that group AB, resulting from the combinations of groups A and B, would be only of the

formula (Ab) (aB). If this were true, the results in Table I could properly be interpreted as crossovers and non-crossovers. The assumption is granted that the original phase was the repulsion phase, that is, that A and b were linked on one chromosome and a and B on the other. Thus the first individuals of group AB which appeared in the phylogeny of the blood groups were undoubtedly of the formula (Ab) (aB). This condition, however, would not prevail long, unless the factors were completely linked, and no further mutations took place.

If any crossing over at all occurred, the new combination (AB) would be formed. Since this would be as stable, once it was formed, as either of the original combinations (aB) or (Ab), it would only occasionally cross back when the opportunity was presented, that is, in combination with a chromosome carrying (ab). The new combination (AB) would thus tend to pile up, and it is this fact that makes the hypothesis of linked factors untenable. How extensively would the combination (AB) pile up? And how would this invalidate the hypothesis of linked factors? Let us examine the material on a frequency basis. Let p equal frequency of A, q equal the frequency of a, r equal the frequency of B, and s the frequency of b. Then $p + q$ equals 1 and $r + s$ equals 1.

Based purely on laws of chance recombination, the combination (AB) should reach a maximum frequency in the general population of pr, as follows:

	A	a
	p	q
B r	AB pr	aB qr
b s	Ab ps	ab qs

The question arises, Would linkage invalidate this? Would (AB) actually reach this frequency, or might it even exceed it?

The piling up of (AB) chromosomes will cease, and a balance between the four combination (AB), (Ab), (aB)

and (ab) will be struck, when the double heterozygotes of group AB consist of as many individuals of the coupling phase (AB) (ab) as of the repulsion phase (Ab) (aB), since these combinations are the only ones in which crossing over can change the factor relationships, and since crossing over in either of these phases results in the production of gametes of the other phase. If the union of the four kinds of gametes in the general population is represented by the usual checkerboard, and the gametic combinations entering into the various individuals are kept in parentheses, to indicate the linkage, the coupling and repulsion phases of group AB may be distinguished. It will be seen that as far as numbers of squares in the checkerboard are concerned, there result equal amounts of the coupling and repulsion phases. But this is without consideration of the frequencies of the various factors concerned. Would this alter the equality? If the letters p, q, r and s are now added to the checkerboard, it will be seen that using the frequency pr for AB, and the corresponding frequencies for the other gametes, the coupling and repulsion phases of the double heterozygotes of group AB will still be equal; that is, the number of individuals of (AB) (ab) will be equal to the number of individuals of (Ab) (aB), because each will occur with the frequency 2 p q r s, as follows:

	AB pr	Ab ps	aB qr	ab qs
AB pr	(AB) (AB) p^2r^2	(AB) (Ab) p^2rs	(AB) (aB) pqr^2	(AB) (ab) $pqrs$
Ab ps	(AB) (Ab) p^2rs	(Ab) (Ab) p^2s^2	(Ab) (aB) $pqrs$	(Ab) (ab) pqs^2
aB qr	(AB) (aB) pqr^2	(Ab) (aB) $pqrs$	(aB) (aB) q^2r^2	(aB) (ab) q^2rs
ab qs	(AB) (ab) $pqrs$	(Ab) (ab) pqs^2	(aB) (ab) q^2rs	(ab) (ab) q^2s^2

Thus, if linkage were concerned, the conditions for the maximum frequency pr of the combination (AB) would be met, quite independently of the frequencies of the

factors concerned or of the amount of crossing-over occurring, the only requirement being the elapsing of sufficient time. In the case of the human blood group this may be safely assumed. The frequencies of the factors concerned in linkage would thus be equivalent, after a balance is reached, to those in independent assortment.

We may then derive in the usual way from the above checkerboard the frequencies p , q , r and s in terms of the percentage of the four groups. These percentages would of course be known quantities in any particular race. This is done briefly as follows:

$$\text{Group O} = q^2s^2$$

$$\text{Group A} = p^2s^2 + 2pq s^2 = s^2(1 - q^2)$$

$$\text{Group B} = q^2r^2 + 2q^2rs = q^2(1 - s^2)$$

$$\text{Group AB} = p^2r^2 + 2pqr^2 + 2p^2rs + 4pqrs = (1 - q^2)(1 - s^2)$$

From the first three equations, p , q , r and s may be calculated in various ways. The most accurate way, as I have previously shown, is as follows:

$$q = \sqrt{\frac{O}{O + A}}$$

$$s = \sqrt{\frac{O}{O + B}}$$

$$p = 1 - q$$

$$r = 1 - s$$

From this it is easy to calculate the frequencies of the gametic combinations, since we have expressed these as pr , ps , qr and qs . By filling in these gametic frequencies in the above checkerboard, we may obtain the expected frequency of any genotype (and so of any phenotype) in any particular population. Using my percentage of the four groups among Americans, we find the frequencies p , q , r and s to be .3, .7, .1 and .9, respectively. The frequencies of (AB) , (Ab) , (aB) and (ab) are thus .03, .27, .07 and .63, respectively.

If we now consider crosses of $AB \times O$, on the basis of linked factors, we find that the AB members of such crosses, even if close linkage occurred, would after all these years of crossing over consist of equal amounts of the coupling and repulsion phases (double heterozygotes), plus some single heterozygotes $[(AB)(Ab)]$,

(AB) (aB)], and some double homozygotes (AB) (AB). Among Americans, the double heterozygotes [(AB) (ab) and (Ab) (aB)] should each occur in 3.78 per cent. of the general population. The single heterozygotes (AB) (Ab) should occur in 1.62 per cent., the single heterozygote (AB) (aB) in 0.42 per cent., and the double homozygote (AB) (AB) in 0.09 per cent.

The above figures give us two separate bases for disproving the hypothesis of linked factors as an explanation of the heredity of the human blood groups. In the first place, if we add up the frequencies of the various genotypes of group AB, we obtain a total frequency of AB of 9.69 per cent. This is much larger than the observed per cent. (4.0), and brings us right back to the original statistical objection to the hypothesis of two factors, linked or not linked. This deficiency of group AB, in comparison with expectation, holds for all races studied, whereas on the triple allelomorph hypothesis the observed proportion agrees closely with the expected.

In the second place, it is seen by examining the expected results of crosses of O and AB on the basis of linkage that they are entirely contrary to the observed results.

From the figures given above we may determine the relative proportions of the various genotypes within the group AB. Using these figures, the expectation in crosses of groups O and AB among Americans is as follows:

Genotype of cross	Proportion of total AB	O	A	B	AB
(AB) (AB) × (ab) (ab)	.009				all AB .0090
(AB) (Ab) × (ab) (ab)	.167		50% A .0835		50% AB .0835
(AB) (aB) × (ab) (ab)	.044			50% B .0220	50% AB .0220
(AB) (ab) × (ab) (ab) }	.780	25% O	25% A	25% B	25% AB
(Ab) (aB) × (ab) (ab) }		.195	.1950	.1950	.1950
Total AB × O	1.000	.1950	.2785	.2170	.3095

The four columns, O, A, B and AB, should thus in linkage show approximately 20 per cent., 28 per cent., 22 per cent. and 30 per cent. of the offspring, respectively. The relative proportions of groups A and B would change somewhat with the race, A being greater in European races and B greater in Asiatic races. Linkage would not, however, produce large numbers of individuals in the two middle columns (non-crossovers) and small numbers in the two outside columns (non-crossovers), as the investigators cited above have thought.

There is thus seen to be no basis for Bauer's hypothesis of linked factors as applied to the human blood groups.

Two other suggestions of different workers may be briefly mentioned.

Wiener, Lederer and Polayes (1930), after reporting the results of the examination of the bloods of 1,334 mothers and 1,462 children, in which they find complete agreement with the hypothesis of triple allelomorphs, suggest a hypothesis to be used if it is necessary to explain exceptions. This hypothesis, which the authors probably do not advance very seriously, assumes the presence of 4 allelomorphs, O, A, B and a new factor D, which determines the presence of both agglutinogens in the cells and thus acts as AB. This can quickly be shown not to explain the recorded exceptions, however, and need not be taken seriously.

Levine has suggested to the writer and to other workers the possibility of non-disjunction as an explanation of the exceptional cases. If I can be convinced of actual exceptions, it is my belief that non-disjunction would be the most reasonable explanation for them. I have yet to find or to be shown a bona-fide exception, however, to the hypothesis of triple allelomorphs, and there can seem to be no reasonable doubt that this hypothesis must stand as the basis of the inheritance of the human blood groups. It is possible that it may be modified by the very occasional occurrence of the phenomena of non-disjunction.

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A LAMARCKIAN EXPERIMENT

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INTRODUCTION

AN experiment begun in 1925 to test out the Lamarckian hypothesis in evolution ran for five years and was abandoned. The Lamarckian hypothesis has many followers, it is superficially reasonable, difficult to disprove, yet it is equally difficult to find facts for its support; facts without a flaw. As Lamarck defines the hypothesis in his "Philosophie Zoologique" four factors are emphasized:

In every animal which has *not exceeded its term of development*, the more frequent and *sustained use* of any organ gradually strengthens the organ, develops and enlarges it and gives it strength proportional to the *length of time* of such use; while the constant *lack of use* imperceptibly weakens it, causing it to become reduced, progressively diminishing its faculties, and ends in its disappearance. . . . Everything which nature has caused individuals to acquire or lose . . . is preserved *by heredity* and passed on to the new individuals which descend from it. (Packard's 1901 Translation.)

If we summarize the factors mentioned above we find:

1. Sustained use or disuse of parts increases or decreases an organ.
2. During the growth period of the organ.
3. Over a period of time; which is interpreted to mean over a series of generations.
4. The changes are inherited.

In the investigation which is described below, factor (1) and (2) can be demonstrated as true. Factor (3) was not tested sufficiently (five years or six generations) and factor (4) was negative. An orthodox Lamarckian would rightly criticize these experiments because factor (3), the time factor, was too short. As the experiment was carefully controlled, as far as it went, it is thought that the conclusions should be recorded.

MATERIAL AND METHODS

Albino rats from the Wistar Institute Experimental colony strain were selected as the medium for the experiment because the generations are short, their vital statistics very complete (Donaldson, 1924), methods for their care available (Greenman and Duhring, 1923), and the dimensions of the bones were capable of statistical treatment.

From these rats the fore limbs were removed, under ether, four to twelve days after birth (Colton, 1929). The rats were raised in special cages which allowed a six foot run (Colton, 1929). The biped rats so produced were bred for six generations.

In the first generation one half of each litter was not operated upon and these constitute the control. Although it was planned to kill the rats when 150 days old, various conditions interfered with this plan in generations beyond the first. Most of the members of the fifth generation were over a year old when killed, because every chance was given them to produce offspring; and it was only when the reproductive period of the females was over that they were killed. This may account for some of the discrepancies between the measurements of the fifth generations and those of the earlier ones.

When the rats were killed the skull and both hind legs were dissected off, boiled in 2 per cent. Star-Naphtha, and the bones cleaned. These were dried at room temperature and then stored away in glass containers until measured.

When the plans for the experiment were under consideration, it was thought that the statistical methods would be simple. The difference between the means of the dimensions of organs of the biped rats and those of the control was to be recorded; and, as a test of reliability of these differences, the differences were to be divided by their standard error. If a difference be over two times the standard error then the difference was to be considered significant.

But all sorts of difficulties arose because rats through life never stop growing and all that time the relative proportions of organs are normally changing. A correction based on body length was first considered; so, because of the unreliability of this dimension, the measurement of a soft part which can never be accurate and which, after the first measurement, can not be verified, the skull length was taken as a base.

When the program was planned, in 1925, the author expected to carry the experiments through at least ten generations. Although the rats bred well through four generations, yet in the sixth only four rats were produced and these like most of the fifth generation refused to breed. It is thought that some dietary lack in the autumn of 1929 was responsible for this failure to breed. No visible sign of a dietary lack was evident. The rats were in fine physical condition. Three female specimens of the fifth generation were submitted to the Wistar Institute for examination. Dr. Donaldson reported as follows:

682—left ovary infected and enlarged—other normal.

683—lung infected, not very badly: probably pneumonia.

672—ovaries seem normal; may be tiny fetus in left horn of uterus near lower end.

673—left ovary and tip of uterus full of pus. Right ovary seems normal; capsule in uterus just beneath ovary appeared hard and granular like cancerous tissue.

It will not be necessary to report in detail the methods used in the measurements of the bones as these were fully discussed in a previous paper (Colton, 1929).

The following measurements were made on each rat:

1. Body length—nose to anus—measured in a special trough.

2. Total length—nose to end of tail—measured in a special trough.

3. Skull length.

4. Skull breadth.

5. Femur length—right and left, right only used in tabulation unless right was injured in preparation and then the left was substituted.

6. Tibia length—right and left, right only used in tabulation unless the right was injured in preparation and then the left was substituted.

7. Tibia breadth.

8. Mesial bend of fibula. In the paper (Colton, 1929) an error is made in reporting the method used. On page nine and in Fig. (1) H, the measurement should be from

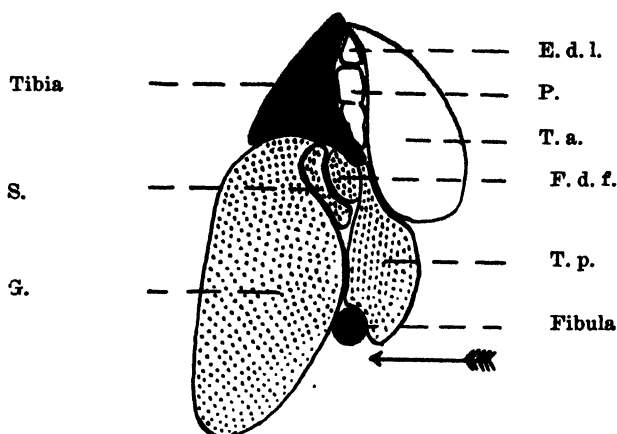


FIG. 1. Diagrammatic cross-section of the right hind leg of an Albino rat showing the relations of the bones and muscles. The bones are black, the flexor group of muscles stippled and the extensor and peroneal groups plain. The arrow points to the direction which the fibula is shifted in the biped rats.

E. d. l. Extensor digitorum longus.

F. d. l. Flexor digitorum longus and fibularis.

G. Gastrocnemius medialis, Gastrocnemius, lateralis, and Plantaris.

P. Peroneal group.

T. a. Tibialis anticus and extensor hallucis.

T. p. Tibialis posticus.

the outer edge of the tibia to the outer, not inner edge, of the fibula.

In compiling the tables of results which are reported below, the bones of each operated rat were compared with a control of equal skull length. This was accomplished through the preparation of curves of femur length on skull length, tibia length on skull length, etc.

By means of these curves, from the femur length of the operated rats, the femur length of the control rat of equal skull length is subtracted.

The same process is repeated for tibia length, for tibia breadth and for mesial bend of the fibula. From these differences, means, standard deviation and standard errors are computed by ordinary methods. These are recorded in Table I. If a difference is twice the standard error it is considered significant.

TABLE I

Bone	Generation	No.	Mean of differences between biped and control	Standard error of difference	Difference divided by standard error
Femur l.	F 1	115	.73 mm.	$\pm .078$	9.3
	F 2	18	.69	$\pm .238$	2.8
	F 3	15	.56	$\pm .199$	2.9
	F 4	26	.71	$\pm .128$	5.5
	F 5	25	.06	$\pm .139$	No Sig.
Tibia l.	F 1	115	.68	$\pm .088$	7.9
	F 2	18	.42	$\pm .098$	4.2
	F 3	27	.47	$\pm .121$	3.8
	F 4	27	.74	$\pm .145$	5.0
	F 5	26	.42	$\pm .081$	5.2
Tibia br.	F 1	114	.18	$\pm .021$	8.5
	F 2	16	.38	$\pm .115$	3.3
	F 3	15	.09	$\pm .042$	2.1
	F 4	27	.26	$\pm .058$	4.9
	F 5	26	.03	$\pm .029$	No Sig.
Fib. mesial bend	F 1	110	.48	$\pm .032$	14.9
	F 2	17	-.02	$\pm .068$	No Sig.
	F 3	15	.25	$\pm .081$	3.1
	F 4	27	.51	$\pm .021$	2.4
	F 5	26	.52	$\pm .049$	10.6

An inspection of Table I shows what is well known; viz., a sustained use of an organ increases the size of the organ during the growth period. The differences of the first generation between the operated and control are

overwhelmingly significant. A further inspection of Table I shows that there is no progressive change to the fifth generation. Therefore, there is no indication that acquired characteristics are inherited.

RESULTS

An inspection of Table I confirms the conclusions of the earlier paper on the difference between the first generation of bipeds and the control. It has an advantage over the earlier results, for it is based on over twice as many rats. It is seen that the femur is, on the average, .73 mm longer than control of same skull length, tibia .68 mm longer than control, tibia breadth .18 mm longer than control. The mesial bend of the fibula is .48 mm more than control. Therefore, a sustained use of these organs increases the size during the growth period. This is, of course, a well known phenomena.

To see if the changes in the bones were due to increases in the muscles, two experiments were performed in which certain muscles of the lower legs were dissected out and weighed wet. In the first experiment, 38 rats biped and 30 control.

The systems of muscles selected were:

A. Flexor group.

1. Gastrocnemius medialis, gastrocnemius lateralis, plantaris and soleus, dissected out and weighed as one.
2. Flexor digitorum longus, and flexor digitorum fibularis weighed as one.
3. Tibialis posticus.

B. Extensor group.

4. Tibialis anticus and extensor hallucis weighed as one.
5. Extensor digitorum longus.

C. Peroneal group.

6. Taken out together, weighed as one.

The weight of each muscle was computed as a percentage of the sum of all the muscles of the lower leg. It will be noted in Table II that in the case of the extensor, peroneal groups and the tibialis posticus increased while the flexor system except the tibialis posticus tended to decrease.

TABLE II

Muscles	Difference in percentage between biped and control	
	60 days	150 days
Gastrocnemius, plantaris, and soleus	- .6	- 1.3
Flexor digitorum longus and f. d. fibularis	—*	- 3.7
Tibialis posticus	+ 39.7†	+ 9.4
Tibialis anticus and extensor hallucis	+ 1.1	+ 2.4
Extensor digitorum longus	+ .8	+ 1.1
Peroneal group	- 5.1	+ 1.9
Number of Rats operated	34	38
Number of Rats control	27	30

* In the case of the small rats it was next to impossible to separate the flexor digitorum longus and flexor digitorum fibularis from the tibialis posticus in a manner sufficiently uniform for statistical treatment. The flexor digitorum longus and f. d. fibularis have not been considered because the figures were so variable.

† In the small rats, part of the flexor digitorum fibularis and f. d. longus adhered to the tibialis posticus. That accounts in part for the large difference.

The experiment was repeated with 60 day old rats, 34 rats operated and 27 rats control, with results similar to the 150 day old rats, with the exception that the peroneal group of muscles showed a decrease. In the small rats it was difficult to separate the flexor digitorum group, fibularis and longus, from the tibialis posticus; so, as the results were irregular the two were added together.

Taking everything, all in all, it is important to note that the tibialis posticus and anticus on the lateral side of the fibula increase while the flexor group on the mesial side of the fibula decrease. This difference in the mus-

cles may account, in part, for the mesial bend of the fibula.

In an earlier report (Colton, 1930) it was shown that an angular bend in the tibia was correlated with the fact that, in walking, the feet of biped rats are spread apart, an adaption to greater stability. A mesial bend of the fibula would be caused by the bend in the tibia. Two factors then explain the bend of the fibula.

CONCLUSION

1. Although change of habit will change the proportion of the bones of the legs of rats, there is no evidence that over a span of six generations that the changes are inherited.

2. The mesial bend of the fibula seems to be due to a relative increase in the tibialis anticus and tibialis posticus, as well as a bend in the tibia, an adaption to greater stability.

3. As far as the Lamarckian factor in evolution is concerned the experiments are negative.

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THE ANTIQUITY OF INSECT STRUCTURES

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ANY warm night in summer time, one may observe those minute Diptera known as punkies, which are so small that they pass without difficulty through the mosquito screen. Mere specks to the naked eye, they are nevertheless more complicated than any automobile, more efficient in flying than any aeroplane. They have muscles, nerves, a blood system, connective tissue, a reproductive system, and a marvelously complex exoskeleton. Their kind existed millions of years before man developed, and who can say that they may not continue to flourish after he has disappeared? Such marvelous little machines are of enormous antiquity, coming down through the ages in variously modified types, but with surprisingly little change in essential characters.

We are all interested in diversity, in progress, in evolution. But it is useful to consider also the stability of living things, the extraordinary fact that in them the most complex chemical substances known retain through vast ages their essential identity. The mammals, which have undergone so many remarkable developments during Tertiary time, are really exceptional. The lower vertebrates, and especially the invertebrates and plants, show a much slower rate of evolution, though it is possible or probable that they too had their time of rapid evolution in the remote past. Considering all the evidence, we are inclined to formulate some general statements, thus:

(1) The rate of evolution in any large group is not uniform; there are periods of relative stability, and periods of comparatively rapid change.

(2) Tertiary time has seen rapid change in the mammalia, but not in the insects, mollusks or flowering plants.

(3) Among the insects, we do indeed find many extinct genera in Tertiary rocks, but these are usually rather closely related to those now living, and the indications are that in at least the majority of cases they have died out, leaving no descendants.

(4) Every new collection of Tertiary insects shows us extinct species of genera still living, so that we are constantly pushing our records of these genera backward into the past, and increasing our estimates of their age. The same, of course, applies to the families.

(5) It is, of course, true that innumerable modern genera are unknown as fossils, but this need not be taken to suggest that they are of recent origin. Considering the small number of fossil insects, compared with those known living, we can not expect to find complete faunae for any periods in the past.

(6) Genera are of different degrees of distinctness. Some, which differ only by a vein in the wings, or a joint in the palpi, may indeed be very modern; for we can often observe these changes taking place as aberrations in existing species. Such genera, having no very fundamental distinctive characters, are likely to be more or less artificial. That is, species showing the characters may have arisen independently a number of times.

(7) Positive evidence of the extinction of genera in various regions is afforded by the fossils. Thus the tsetse flies (*Glossina*) have entirely died out in America, leaving no descendants. The family Nemopteridae once lived in North America, but has now disappeared from this continent.

(8) Probably the best evidence concerning the actual evolution of insect genera in Tertiary time may be derived from oceanic islands, especially when it is possible to form some idea of their antiquity. The Hawaiian fauna is especially instructive in this respect.

In order to present concrete evidence, we offer descriptions of several quite diverse insects, collected by Pro-

fessor Junius Henderson and Mr. John Byram in the rocks of Green River age in western Colorado. This deposit is Eocene, and is the oldest known, which contains an essentially modern (that is, like the modern) insect fauna of any size. The examples selected belong to five different orders.

LEPIDOPTERA

Tillyard has shown that the supposed Mesozoic Lepidoptera do not belong to that order. In the Tertiary rocks, Lepidoptera are scarce, owing to the fact that they are less likely to be preserved in recognizable form than any other winged insects. There are various records from the European Oligocene, and in America from the Miocene of Florissant. A beautifully preserved moth comes from the Oligocene of the Isle of Wight, and was described by Butler in 1889 as *Lithopsyche antiqua*, a new genus of Euschemidae. It has no striking peculiarities, but combines the characters of several living genera, which at the present day inhabit the Malay region. Were the climate such as to permit these moths to live to-day in Britain, the British genus or genera would most probably differ from those of the Malay Archipelago. Thus we need not suppose *Lithopsyche* to be the ancestor of *Calospila*, *Craspedopsis* and *Mniocera*, but merely that it died out in Europe when the environment changed. It does not appear evident that *Lithopsyche* is any more primitive than its living representatives. There is, however, a certain amount of evidence that the more primitive or the less specialized Lepidoptera, of families still existing, were proportionately more numerous in the faunae of Tertiary time than at present. Thus the senior author has in recent years had occasion to describe two new genera of fossil moths, one from the Oligocene of the Isle of Wight, the other from the Miocene of Florissant. Both are Cossidae, a family which all lepidopterists now place low in the series. In modern faunae, the chances would be very greatly against any two moths,

thus accidentally fossilized, being both Cossidae. In the Burmese Amber, which probably comes from low down in the Tertiary, a single moth has been preserved, and it belongs to the exceedingly primitive genus *Micropteryx*. Nevertheless, such moths still live in various parts of the world, so the fossils take us no nearer to the stem of the Lepidoptera than the oldest existing types.

With the possible exception of the specimen from Burmese amber, the moth now described appears to be the oldest Lepidopterous insect so far known.

CHIONAEMOPSIS new genus

Anterior wing only known. A rather small moth, the shape of the wing, and the pattern of dark cross-bands, strikingly like that of the genus *Chionaema*, which has numerous species in the tropics of the Old World. The venation, however, is conspicuously different, though in the fossil it is not as well preserved as could be wished. Another similar insect of quite different relationships is the Australian *Chrysonoma argutella* Zeller, of the family Oecophoridae. This has, as in the fossil, four vertical dark bands crossing the wings, the fourth forked above to make a Y. The venation of the Oecophoridae also agrees much better with the fossil; thus in the course of the subcosta, the radius forking before the middle of the wing, and its lower branch (radial sector) again forking in the region of the third band. In the figure, every effort has been made to show the venation of the fossil as it is, but it is so faint in many places that a really adequate drawing can not be made. It is not possible to make out the definite outline of scales, but small objects scattered over the dark areas appear to represent them, and there is an evident fringe.

The same pattern of four cross-bands appears also in *Iphierga* of the Tineidae, and there are reasons for believing it to be a very ancient one.

CHIONAEMOPSIS QUADRIFASCIATUS n. sp.

Anterior wing about 13 mm long (base missing); from first band to apex 10 mm; width nearly 5 mm; four dark cross-bands, the fourth forking near costa, as shown in figure.

Green River Eocene, Colorado. Station 16, on Parachute Creek, 1923 (Henderson and Byram).

HOMOPTERA (FULGOROIDEA)

Protoliarus amabilis n. sp. A beautifully preserved upper wing or tegmen, showing every detail of the venation and marking. The wing is 7.6 mm long and 2.8 mm wide, thus considerably larger than *P. humatus* Cockerell. It also differs in the costal nervure, and in having the tegmen more broadly and obtusely rounded at end. This genus closely resembles in a general way many now living, but its exact relationships are rather obscure, as has been explained in Proc. U. S. National Museum, Vol. 64, Art. 13 (1924) p. 8.



PHOTOGRAPHS

- | | |
|---|-------------------------------|
| 1. <i>Chionaemopsis quadrifasciatus</i> . | 3. <i>Tryphon amasidis</i> . |
| 2. <i>Protoliarus amabilis</i> . | 4. <i>Chilosia scudderi</i> . |
| 5. <i>Adelocera perantiqua</i> . | |

For the photographs we are indebted to the kindness of our colleague Dr. Ross Whitman.

Superficially the insect resembles *Oliarus*, and the size agrees with the fossil *Oliarites terrentula* (Scudder), but it is distinct by the clouded wings (wholly clear in Scudder's species) and the details of the venation.

The stem of the media before the first fork (where there is a vertically elongate dark spot) is about 1.9 mm long; from the fork to the end of the cell of which it is the base is 2.7 mm; the oblique vein to cubitus meets the fork of the latter.

In the existing fauna, these Fulgoroids present an amazing complex of related genera, new ones being continually discovered. It does not appear evident that *Protoliarus* is an ancestor of any of them, but the climate of the Rocky Mountains has changed and the numerous Fulgoroids, of tropical aspect, have disappeared.

Green River Eocene; Station 16, Parachute Creek, Colorado, 1923 (Henderson and Byram).

HYMENOPTERA (ICHNEUMONIDAE)

When we consider the numerous adaptive peculiarities of the parasitic Hymenoptera, and their often remarkable habits, it is natural to suppose that they are of relatively recent origin. This is not at all the case, and the Ichneumonidae of the Eocene are essentially at the same evolutionary level as those of modern times. The species now offered as an example is placed in *Tryphon*, as understood in the broadest sense, because the fine details of structure relied on for generic characters at the present time are not visible. Had we the complete insect, it presumably would fit easily into the modern classification.

Tryphon (s. lat.) *amasidis* n. sp. Length slightly over 6 mm, width of thorax 2 mm, of abdomen 1.6 mm; anterior wing 5 mm long. Wings hyaline, with pale nervures and stigma; antennae very pale; legs practically colorless as preserved; head and thorax dark, probably originally black; abdomen brown, the margins of the tergites

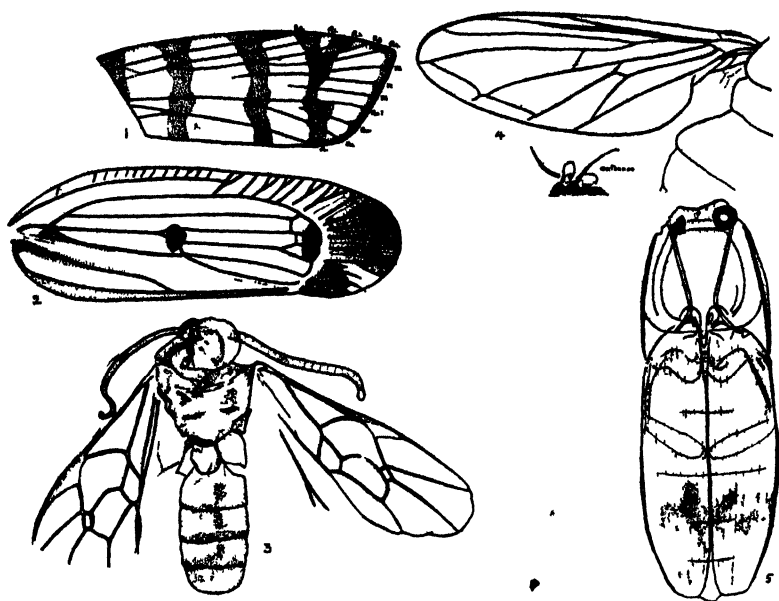
distinctly infuscated. Anterior wings with areolet elongated, four sided, the apical side smallest; lower end of basal nervure basad of nervulus by a distance fully equal to half length of latter. The peculiar shape of the thorax appears to be due to crushing. Compared with typical *Tryphon* the areolet is much longer (in *Tryphon* often practically square) and the basal nervure is much more remote from the nervulus. Another striking feature is the extremely oblique nervulus (the lower end apical), much in the manner of *Tryphon rutilator* (L), which is the type of the genus *Tryphon*. The species from the Florissant Miocene, described by Brues under *Tryphon*, seem to be doubtfully congeneric. In this key he separates *T. cadaver* from *T. peregrinus* by the shape of the stigma, but the latter, as figured, does not possess the kind of stigma indicated in the key. Of the Florissant species, ours appears to be nearest to *T. cadaver*, but that is smaller and has a much shorter marginal cell. Brues says *T. cadaver* has an areolet, but the figure shows none. In the key to Isle of Wight (Oligocene) Ichneumonidae, *T. amasidis* runs directly to *Lithapectis fumosus*, which has quite the same kind of areolet, but differs in many respects.

The insect was presumably parasitic on some sawfly, and so is named after a genus of sawflies present in the Green River shales.

Green River Eocene, Station 26, Roan Plateau, 1923 (Henderson and Byram).

DIPTERA (SYRPHIDAE)

Chilosia scudderi n. sp. Length 7 mm; width of abdomen not quite 3 mm, length of anterior wing about 7.2 mm; head and thorax black; wings hyaline, slightly greyish; abdomen broad and short, pallid, with the hind margins of the tergites broadly infuscated; legs pallid so far as visible. The following measurements are in microns: length of costal cell 2860; base of costal cell to



DRAWINGS

- | | |
|---|-------------------------------|
| 1. <i>Chionaemopsis quadrifasciatus</i> . | 3. <i>Tryphon amasidis</i> . |
| 2. <i>Protoharus amabilis</i> . | 4. <i>Chilosia scudderi</i> . |
| 5. <i>Adelocera perantiqua</i> . | |

end of subcostal 4900; length of anterior cross-vein 400; anterior cross-vein to base of first submarginal cell 900, to base of discal 800, to apex of discal 1960; last posterior cell on axillary 460; length of arista 1000.

This is evidently Scudder's *Chilosia* sp. figured in Tertiary Insects of North America, plate 9, f. 26, and discussed on p. 561. Scudder's specimen came from Green River, Wyoming.

This appears to be congeneric with *C. miocenica* Cockrell, from the Miocene of Florissant, but the marking of the abdomen suggests that it is not a genuine *Chilosia*. However, the Asiatic *C. plumbiventris* Brunetti has black bands on the abdomen.

The fly is in no sense more primitive than many now living.

Station 22, Roan Plateau, Colorado, 1923 (Henderson and Byram).

COLEOPTERA (ELATERIDAE)

Adelocera perantiqua n. sp. Length 8.5 mm, width across elytra 3 mm; color pale (eyes black), with two suffused dark marks on each elytron, giving the effect of broad transverse dusky bands. The elytral striae are as usual, and the prosternal spine is long and acute. The elytra are about 5.5 mm long. Antennal grooves distinct.

It is about the size of the living *A. discoides* Web., but the blotched elytra rather suggest *A. marmorata* Fab. The elytral pattern and general appearance are suggestive of our common *Drasterius elegans* Fab., which, however, belongs to a different group.

Adelocera has many species in the Nearctic and Palaearctic regions to-day. The European *A. fasciata* (L.) has the elytra much as in the fossil, but it also has spots on the thorax.

Station 20, Roan Plateau, Colorado, 1923 (Henderson and Byram).

SOME FACTORS AFFECTING THE DISTRIBUTION OF AND VARIATION IN NORTH AMERICAN ECTOPARASITES

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THE distribution of the groups of higher vertebrate animals and their variation and speciation in Nearctic North America have long been subjects for fruitful research. Associated with these higher vertebrates are host-infesting ectoparasites of several groups, the distribution of which is dependent to a greater or less degree upon the distribution of their hosts. Their distribution, therefore, offers a problem somewhat more complex than that of their free-living hosts.

Dr. Karl Jordan (1929) has presented a most excellent paper on the problems of distribution, variability and variation in North American fleas. He found that there were 131 described species in America north of Mexico. This number probably represents at least two thirds of all those that exist in this territory.

The outstanding fact in regard to the relationship of distribution to variation is that the latter, as expressed in numbers of genera and species, is far more pronounced in the western part of the continent than in the eastern part. Dr. Jordan gives the key to the situation in the following words: "Among the 31 species so far found in the Eastern States there are 17 which also occur in the West or at least are there represented by special subspecies, leaving 14 which are restricted to the East, but probably extend on to the Central plains, at least in the North. Some of these 14 are purely Northern forms, being only known from New England and the neighboring States; others are of wider southward distribution. In the Western area, on the other hand, the number of indigenous species confined to the West, but partly de-

scending eastward into the foothills, is 90, more than six times as many as in the Eastern States."

Dr. Jordan has discussed various factors, which may have brought about this paucity of genera and species in the eastern part of the continent as compared with the western part. Among these are mentioned the fact that apparently but little collecting has been done in the southeastern part of the United States and also that the summer climate of the low levels of the East is adverse to fleas in general. Yet the chief reason for the differences in the diversity of the flea faunas of the East and West is attributed to glaciation. He states: "In the glacial period life was practically destroyed in the Northern Atlantic States, whereas in the Pacific half of the continent glaciation was less complete, so that life could persist in many cases. This, no doubt, accounts to a large extent for the greater abundance of species in the West."

The present writer, having collected and studied ectoparasites from nearly all sections of the United States, has long noted this greater diversity of the fauna in the West. It is not at all confined to fleas but appears to be more pronounced in this group than in the host-infesting mites, the biting lice, or the sucking lice. Yet in the ticks, a group in which much of the time of the individual parasite is spent detached from its hosts, there is a condition paralleling that found in the fleas. Thus in the genus *Dermacentor*, a genus particularly well represented in North America in comparison with the other continents, there are present in the western part of the United States nine species, while in the eastern part there are only three and only one of these is abundant.

The present writer would attribute the greater diversity of the flea and tick faunas of the West, first, to the presence of natural barriers in the form of high mountain ranges, and secondly, to the diversity of climate. Along the northern border of the United States there are

three closely related species of *Dermacentor*, so closely related in fact that they were at first confused with one another. One of these, *D. occidentalis* Neumann, is found only west of the Cascade Mountains; another, *D. andersoni* Stiles, is found only between the Cascades and the plains; and the third, *D. variabilis* Say, is found only east of the arid plains, except for an isolated area along the Pacific slope of southern Oregon and California. That this distribution is brought about chiefly on account of natural barriers and different climatic zones is strongly indicated. The Rocky Mountain spotted fever tick, *Dermacentor andersoni* Stiles, originally was confined to the area between the Rocky Mountains on the east and the Cascade and Sierra Ranges on the west; but since the introduction of domestic animals it has spread considerably to the eastward but has never crossed the plains. There can be but little doubt that large numbers of *D. andersoni* have been carried into the plains and to the eastward in the past, and also that large numbers of *D. variabilis* have been carried into all regions of the West. Consider the number of these ticks that must have been taken into the West by the pioneers with their horses and cattle to say nothing of their dogs. It is stated that at times some of the roads to the western states were clogged with hundreds or thousands of these immigrants and their domestic animals. Yet with these ideal conditions for the spread of the eastern *Dermacentor*, it does not seem to have obtained a foothold in the West except along the southern part of the Pacific slope.

Variation and speciation have, in fact, taken place to a much greater degree in the western part of North America in groups other than ectoparasites. For example Howell (1929) finds that in the chipmunks (*Tamias* and *Eutamias*) there is but one species with five races in the eastern part of the continent; while there are sixteen species representing sixty races in the western part. Then there is that classical example of the song sparrow.

According to recent authorities there are no less than thirty-three races, or subspecies, of this common North American bird. Yet of this large number only four races are found east of the Rocky Mountains; while California alone has ten resident races. Chapman (1920) and others attribute this condition of affairs, and doubtless correctly, to the presence of barriers in the West and to the great diversity of climate, which in turn is dependent largely upon these barriers.

The apparently uneven and unusual way in which the flea, *Pulex irritans* Linnaeus, has distributed itself in the United States has been mentioned by Howard (1896) and Jordan (1929). However, the distribution of this species has not been ascertained with sufficient accuracy in the past. The unusual thing about its distribution has to do rather with its sparseness in certain large areas than its absence in them. Yet certain areas exist in which repeated search for this flea has failed to reveal its presence. In order to get a more complete and up-to-date picture of the distribution of this species, there are here given in tabular form all locality records, based upon specimens determined by specialists, that are in the files of the United States National Museum and of the United States Bureau of Entomology, particularly in the Bureau's Division of Insects Affecting Man and Animals.

AUTHENTIC RECORDS OF THE OCCURRENCE OF *Pulex irritans* LINNAEUS
IN THE UNITED STATES

Arizona: Madera Canyon, Santa Rita Mountains, June 19, 1898; Omaha, July 28, 1928; Phoenix, May 10, 1930.

Arkansas: Imboden, 1928; Georgetown, April 27, 1921.

California: Lakeside; Alameda County; Carbon Canyon, Puente Hills, January 23, 1926; San Diego, December, 1896; Azusa, July 23, 1894; Redwood Creek, Humboldt County, June 17, 1903; San Francisco, October, 1907; Humboldt County, December 1, 1927.

Colorado: Larimer County, November 15, 1926.

District of Columbia: Washington, June 25, 1909 (by W. L. McAtee).

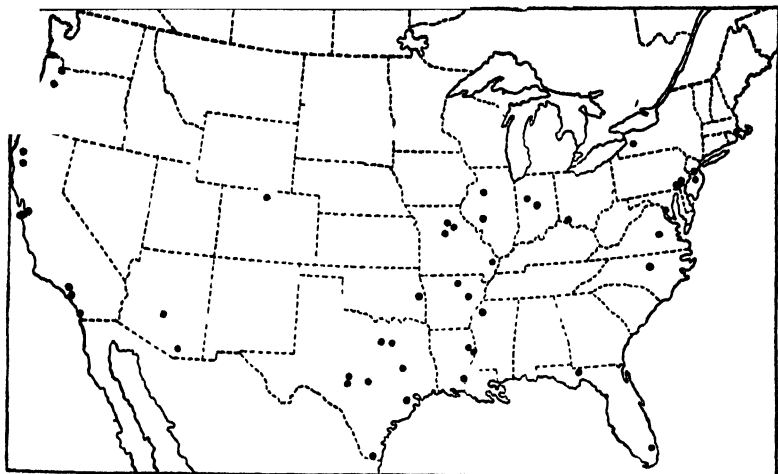
Florida: Little River, November 30, 1912, and December 20, 1913; Quincy, June 7, 1915.

Illinois: Elmwood, July 2, 1914; Winchester, 1921; Kempsville, April 28, 1921.

- Indiana: Frankfort, April 29, 1914; Greenwood, June 19, 1922; locality (†), May, 1928.
- Louisiana: Port Barrie, April 26, about 1915; Tallulah, May, 1918; Mound, June 17, 1918; locality (†), May, 1928.
- Massachusetts: Fall River, August 16, 1921.
- Michigan: Locality (†), 1921.
- Mississippi: Dundee, April 12-15, 1921.
- Missouri: Gilliam, August, 1914; Mexico, April 11, 1921; Atlanta, June 21, 1921; Charleston, July 1, 1921; locality (†), May, 1928.
- Nebraska: Locality (†), fall, 1926.
- New Jersey: New Brunswick, September 18, 1916; Browns Mills, May 5, 1916.
- New York: Springville, November 7, 1921.
- North Carolina: West Raleigh, April 8, 1915.
- Ohio: Cincinnati, June 28, 1915; locality (†), June, 1923.
- Oklahoma: Wister, July 8, 1904.
- Oregon: Portland, 1903; Albany, June 20, 1915; locality (†), October 23, 1928.
- Pennsylvania: Oxford, June, 1912; Chadd's Ford, April, 1921.
- Texas: Brownsville, May 7 and 18, 1904, July 6 and 9, 1904, and July 5, 1895; locality (†), February 10 and 20, 1897; Dallas, September 1, 1915 (questionable identification), May 27, 1905, April 1, 1907, May 4, October 16, October 18, and November 21, 1917, and June 14, 1918; Concan, December 7, 1917; Uvalde, May 31, 1918; College Station, June 3, 1918; San Antonio, summer, 1919; Sandy Point, April 17, 1920; Ft. Worth, April, 1927.
- Virginia: South Richmond, July 6, 1917.
- Wyoming: Locality (†), November 27, 1927.

In addition to these seventy-one records which come from twenty-three states and the District of Columbia, there are many others that are not based upon specimens determined by specialists. These other records are from the same general areas as are covered by the valid ones. Doubtless most of them refer also to *Pulex irritans* Linnaeus.

If we mark on a map the definite locality records, we get a distribution as indicated on the accompanying map. Each dot represents a known locality record. In some instances definite records were obtained for certain states but not for a locality in the state. These have not been marked on the map. Thus there is a record for the state of Michigan, another for Nebraska, and also one for Wyoming, but, since the locality is not known in



Map of the United States showing the distribution of *Pulex irritans* Linnaeus, as indicated by the records (based upon specimens) in the United States National Museum and the United States Bureau of Entomology.

each of these cases, the records could not be given on the map.

Three large areas are to be noted on the map from which we have no records or but very few of them. First, there is that large area between the Rocky Mountains on the east and the Sierra and Cascade Ranges on the west. This area includes the Basin States of Idaho, Nevada and Utah and much of the surrounding territory. Then there is the Great Plains area adjacent eastward of the Rocky Mountains. Finally, there is the southern coastal plain section of the South Atlantic States. Can the absence of records or scantiness of the same in this southern coastal plain section be attributed to lack of search for this flea, or to a probability that it is not being reported by entomologists in this as in the other three areas mentioned? There has been much collecting of ectoparasites in the Basin States area and also in the southern coastal plain section of the South Atlantic States. In fact from these areas both the Bureau of Entomology and the National Museum have an abun-

dance of records of other ectoparasites, including many flea species. In the Great Plains area but very little collecting has been done, and since this area is so sparsely settled one would not expect many reports from the inhabitants.

The absence of records from the Southern Appalachian section is probably due to the lack of collecting. At any rate, for the present, this section should be left out of consideration, as our knowledge of its ectoparasitic fauna is too limited. Dr. Jordan (1929) predicts that *Pulex irritans* will be found to occur in this section.

Since coming to Washington in 1919 the writer has been repeatedly reminded of Dr. Howard's (1902) statement that the fleas sent in for identification from the eastern cities are not *Pulex irritans* Linnaeus but either the cat flea or the dog flea. Not only has the present writer never received in all the eleven years of his connection with the Federal Bureau of Entomology a single specimen of *Pulex irritans* from the big cities of the East, but in his extended field work over a period of several years in the eastern parts of the states of Maryland, Virginia and North Carolina he has never taken a specimen of *Pulex irritans*.

About Washington, D. C., fleas are abundant but, as Dr. Jordan has noted, the number of species is not large. In the low swampy lands of eastern Virginia and North Carolina a month's survey work on ectoparasites by the writer and Charles East during the height of the flea season (July, 1928) revealed only four flea individuals. Two of these were *Echidnophaga gallinacea* Westwood on a rat, one was a species of *Ceratophyllus* on a rat, and the other was on a rat but escaped before being determined. Thus the absence of *Pulex irritans* in this section is attributed to adverse conditions that affect fleas in general. This does not hold in the least, however, as far as the District of Columbia is concerned, for the fleas are abundant in and about Washington. Thus out of thir-

teen specimens of one of our most common mammals, the white-footed mouse, *Peromyscus leucopus*, taken near Washington, eight were found to be infested with fleas; while out of eighteen specimens taken in the low swampy districts of eastern Virginia and North Carolina not a single one had as much as a single flea.

The scarcity of certain flea species in eastern Virginia and North Carolina may be attributed to the scarcity of their favored or normal hosts, but this explanation would not apply to the distribution of the majority of them. This section is very low and flat and most of it during some time of the year is flooded. The rainfall, however, both in total annual precipitation and in its seasonal distribution, is very nearly the same as at Washington, D. C. The surface water, however, does not drain off but accumulates in a soured mixture of peaty composition over much of the area. Experimentally such conditions have been approximated in the water treatment recommended by the Bureau of Entomology for the control of fleas, *i.e.*, the flooding of the infested soil with water, not once but several times, in order to destroy the flea larvae. Such treatments have proven effective against the human flea and dog and cat fleas.

What the writer has observed in regard to the paucity of fleas in his survey of ectoparasites of birds and mammals in the lowlands of eastern Virginia and North Carolina is in accord with the observations of Pearse in Africa. Pearse (1928) found that in Nigeria fleas were much more abundant in the section of that country where the climate was dry. He states: "Fleas flourish best on hosts which live in a dry climate and have a more or less permanent home, such as a burrow or a human habitation."

Although the moisture in the soil and the presence of surface water at frequent intervals on much of the area should account for most of the sparseness of the flea population in the section under consideration, yet there is also another factor to be considered. It is this: The

mammal population as a whole is inclined more to aquatic or semi-aquatic habits. In much of this section, instead of the cottontail rabbit, which avoids the water at all times, there is present the marsh rabbit, *Sylvilagus palustris palustris*, which is semi-aquatic, frequently taking to the water like a true water species.

Pulex irritans is a flea that thrives in association with man, although it may possibly not have man as its most favored host. It is frequently found on domestic animals, particularly pigs. Because of this close association with man and possibly with pigs also, but few of these fleas would be found in districts where human habitations are scarce. This would in part explain the absence or scarcity of *Pulex irritans* in the Great Basin and over the Great Plains. Excessive dryness would be particularly detrimental to the larvae of those species that normally infest nests of hosts that are built above the ground. This would not be true, however, of fleas on certain burrowing mammals, as they are known to thrive in desert conditions. Does *Pulex irritans* breed on any burrowing animal that occurs in our country? An answer to this question might help us understand better its distribution in the United States.

SUMMARY

1. Ectoparasites that spend a part of their life detached from their hosts are affected not only by most of the factors that determine the distribution of their hosts but by many others that affect them as independent arthropods during their free-living periods.

2. The much greater diversity of species and genera in the western part of North America appears to depend fundamentally upon the presence of natural barriers in the form of high mountain ranges and upon the diversity of climate which is largely determined by the presence of these mountain ranges.

3. The distribution in the northern part of the United States of three closely related ticks of the genus *Dermacentor* is discussed.

4. Records of the occurrence of *Pulex irritans* Linnaeus in the United States are published, and the peculiar distribution of this parasite of man is considered.

5. The presence or abundance of *Pulex irritans* Linnaeus in any region of the United States appears to depend in part upon the following:

(a) the proper moisture content in the soil and the absence of surface water on top of the soil during the period of its larval development,

(b) the absence of extremely low temperatures,

(c) the presence of human habitations,

(d) the presence of hosts other than man, these hosts for a particular region not being known at the present,

(e) the habits of all hosts (including man) in the region under consideration.

6. A study of the records of the occurrence and abundance of fleas in general and of *Pulex irritans* Linnaeus in particular would appear to indicate that the abundance of individuals is largely independent of the following factors;

(a) total annual precipitation,

(b) humidity during adult state.

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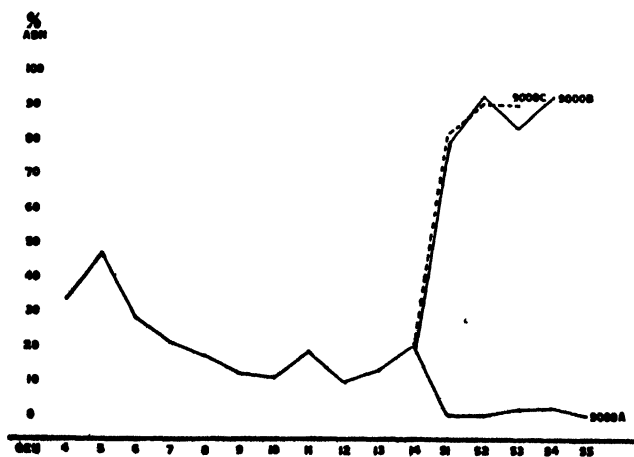
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SHORTER ARTICLES AND DISCUSSION

THE EFFECTS OF SELECTION ON EYE AND FOOT ABNORMALITIES OCCURRING AMONG THE DESCENDANTS OF X-RAYED MICE

IN 1921 the writer and Bagg discovered certain abnormalities of the eyes and feet in mice. These particular genetic abnormalities have occurred only among the descendants of certain animals which as adults (non-pregnant females and males) were treated with light doses of X-rays over the whole body. A series of papers dealing with the various abnormalities from genetic and anatomical points of view are included in the bibliography of this note.

The object of the present communication is to record the results of selection within the abnormal race as it is at present being carried on. It should be recalled that whereas Bagg has at present animals descended without an outcross from the original abnormal, the mice which provide the primary data incorporated in this note are the result of either one or two outcrosses with a normal albino race followed by close (brother to sister) inbreeding and, in certain of the lines, selection of the abnormal individuals as parents. The ancestry of most of these



mice (the stock giving rise to lines 9,000A, 9,000B and 9,000C) is described in some detail by Murray (1928). The 9,000 lines are the result of two outcrosses of the abnormality followed by isolation through inbreeding. The other, line 700, is the result of only one outcross followed by inbreeding.

The derivation of the three 9,000 lines by selection is shown in Fig. 1. The first fourteen generations include the material described by Murray (Gen. 4-14); while the selection generations included in this note are designated as S_1 - S_6 . The details of the three lines will be given below.

Line 700, not shown in the figure, is the result of an attempt to isolate the eye abnormality from the various foot abnormalities. During the period from 1929 to the present year, inclusive, 355 animals have been recorded in it. These animals are distributed over four generations as follows, as regards eye abnormalities:

Generation	Right eye abnormal	Left eye abnormal	Both eyes abnormal	Total abnormal	Normal	Per cent. abnormal
I	35	33	2	70	9	88.6
II	50	36	11	97	7	93.2
III	41	43	17	101	6	94.3
IV	25	26	11	62	3	95.3
Grand total	151	138	41	330	25	92.9

In an earlier paper (1924) the writer and Bagg found by macroscopic examination approximately 81.8 per cent. of the progeny of abnormal-eyed individuals to be abnormal. It is evident that distinct progress is being made by selection in increasing the percentage of abnormal-eyed individuals among the progeny. There has also been, among the abnormal, an increase in the percentage of mice with both eyes abnormal. This percentage, in successive generations, has been 2.8, 11.3, 16.8 and 17.5, respectively.

There have been among the 355 mice only two foot abnormalities; ♂ 446 with right eye and right hind foot abnormal in the first generation, and ♂ 709 with left eye and right anterior foot abnormal in the fourth generation. The percentage of foot abnormal is thus 0.59. It is doubtful whether they will ever be entirely eliminated from the strain by selection, but their reduction to this point is interesting. Bagg (1929) reports in a population of 5,200 mice the occurrence of foot abnormalities in 432, or 8.3 per cent. Murray (1928) in 3,069 mice found 565 young with abnormal feet, a percentage of 18.4. There has, therefore, in the "700" stock, been definite progress towards decreasing the percentage of foot abnormalities.

The three "9,000" selection lines A, B and C were started for different purposes from a single original stock.

The line 9,000A was a reverse selection in the direction of the normal type. In the last five generations the following result has been obtained.

Generation	Normal	Abnormal
A ₁ -S ₁	44	0
A ₂ -S ₂	40	0
A ₃ -S ₃	55	1
A ₄ -S ₄	107	2
A ₅ -S ₅	43	0
Grand total	289	3

In the total number only 1.03 per cent. is abnormal.

The three abnormal individuals are

♀ 1,088 normal eyes and abnormal left posterior foot,

♀ 1,262 normal eyes and abnormal left posterior foot, and

♂ 1,469 abnormal right eye and abnormal left anterior foot.

There is, therefore, in this line only one animal with abnormal eyes out of the total of 292. This percentage of 0.34 is interesting and in marked contrast to the 92.9 per cent. eye abnormalities obtained by selective breeding in line "700."

Line 9,000B is a selection for high number of foot abnormalities especially in the anterior feet. The last four generations have given 85.2 per cent. abnormal as follows:

Generation	Foot abnormality in anterior feet only	Both anterior and posterior foot abnormalities	Foot abnormality in posterior feet only	Total abnormals	Total normals
B ₁ -S ₁	18	9	2	29	8
B ₂ -S ₂	19	17	1	37	3
B ₃ -S ₃	48	53	10	106	21
B ₄ -S ₄	13	8	2	23	2
Grand totals	93	87	15	195	34 (85.1 Per cent.) (abnormal)

Line 9,000C is a selection for high number of foot abnormalities especially in the posterior feet. This line is less fertile than either line 9,000A or 9,000B. In the last three generations it has given a total of 136 animals distributed according to the following table.

Generation	Foot abnormality in anterior feet only	Both anterior and posterior foot abnormalities	Foot abnormality in posterior feet only	Total abnormalities	Total normals
C ₁ -S ₁	4	10	9	23	5
C ₂ -S ₂	11	23	12	46	5
C ₃ -S ₃	10	30	11	51	6
Grand totals	25	63	32	120	16

The total shows 88.2 per cent. of the animals with abnormal feet.

Line 9,000B shows 84.1 per cent. animals with anterior feet abnormal and 75 per cent. with posterior feet affected. Line 9,000C has 84.6 per cent. with abnormal anterior feet and 85.5 per cent. with posterior feet affected. Leaving out individuals that have abnormalities in both anterior and posterior feet, line B has 86.1 per cent. of its abnormalities in the anterior feet only. Line C analyzed similarly shows 43.8 per cent. Some progress in the direction of the selection has been made but it is not as extensive or as striking as is the progress made in the total percentage of foot abnormalities. This for the two lines is 86.3 per cent. and is an interesting contrast with line "700" with 0.59 per cent. and line 9,000A with 1.02 per cent.

The distribution by feet of the abnormalities is also a matter of interest. Compared with the findings of Murray and of Bagg this is as shown in the following table.

It will be noted that Murray's figures and those given by Line 9,000B show a clear excess of left anterior feet; while Bagg's total shows an excess of left posterior feet. This demonstrates an interesting genetic difference which is very likely due to modifying factors in the two strains.

Author	Total number of abnormal feet	Right anterior	Left anterior	Right posterior	Left posterior
Murray, 1928	726	154	277	132	163
Bagg, 1929	510	29	128	149	204
Little, 1931,					
Line 9,000B	421	126	161	63	71
Line 9,000C	284	68	75	75	66
Grand total	1941	377	641	419	504

Murray's mice and the line 9,000B mice show a total of 718 abnormal anterior and 429 posterior feet; while Bagg's mice show 157 anterior to 353 posterior feet abnormal. Line 9,000C totals 143 abnormal anterior feet and 141 abnormal posterior feet.

A comparison between right and left sides of the body shows the following results:

Author	Right	Left	Per cent. left
Murray, 1928	286	440	60.6
Bagg, 1929	178	332	65.1
Little,			
Line 9,000B	189	232	55.1
Line 9,000C	143	141	49.7
Grand totals ..	796	1145	58.9

From these figures it would seem that selection in line 9,000C has increased significantly the proportion of right foot abnormalities. There appears to be an inherent tendency in the figures of Murray, of Bagg, and of line 9,000B to produce a significant excess of abnormal left feet. This is to some degree being overcome in line 9,000C.

The above results demonstrate clearly

(1) That it is possible within the abnormal stock by selection to develop strains differing greatly in the percentage of animals showing eye abnormalities (92.9 and 0.34 per cent.);

(2) That it is possible within the abnormal stock to develop by selection strains differing greatly in the percentage of foot abnormalities (88.2, 85.1 and 0.59 per cent.);

(3) ~~That~~ it is possible by selection to develop within this material a strain high in eye abnormalities (92.9 per cent.) and low in foot abnormalities (0.59 per cent.);

(4) ~~That~~ it is possible by selection, but with more difficulty than in the foregoing cases, to progress in the direction of separating a line high in anterior foot abnormalities (86.1 per cent.) from a line high in posterior foot abnormalities (56.2 per cent.);

(5) ~~That~~ modifying factors appear to play an important rôle in determining the antero-posterior and right-left relationships and incidence of the abnormal feet.

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A POSSIBLE EXPLANATION OF THE APPARENTLY IRREGULAR INHERITANCE OF POLY- DACTYLY IN POULTRY

IN a recent paper by Punnett and Pease (1929) the known facts concerning the inheritance of polydactyly in poultry were brought together and analyzed. It was fairly conclusively demonstrated that a single factor was responsible for the development of extra toes, and certain families gave results in close agreement with Mendelian expectation, with the extra-toed (E.T.) condition dominant over the normal four-toed (n.e.) condition. In many families, however, four-toed (n.e.) birds appeared in F_1 between homozygous E.T. and n.e. birds, and an excess of normal birds was found in F_2 and backcrosses. Where normal birds obtained in F_1 were tested, they were found to carry the E.T. factor.

These irregular results were ascribed to the effect of factors inhibiting the development of extra toes. The observation that a bird with extra toes might carry factors causing an excess of normal birds among his progeny made it difficult to devise a simple factorial scheme to explain all the results.

The object of the present note is to suggest that these facts are consistent with Fisher's (1928) theory that dominance is controlled by genetic factors.

On this view three main types of behavior may be expected:

(1) Normal Mendelian behavior with complete or nearly complete dominance of the polydactylous condition. This will occur when both parents carry factors favoring dominance of E.T. (hereafter referred to as dominance factors).

(2) Abnormal behavior with normal four-toed birds occurring in F_1 , and normal birds in excess of the proportions expected when polydactyly is completely dominant, in F_2 and backcrosses and a corresponding proportion of normal birds behaving as heterozygotes. This will occur when one parent lacks some or all of the dominance factors. It may be either parent which lacks dominance factors, but heterozygotes lacking all the dominance factors should be four-toed (normal).

(3) Normal behavior, with complete or nearly complete dominance of the four-toed condition. This will occur when neither parent carries dominance factors.

The data assembled by Punnett and Pease may now be examined to see how far they fall into these classes.

(1) Normal behavior, with polydactyly dominant. The best example of this is the light Dorking $\times$ Buttercup cross made by Punnett and Pease (pp. 343-344). In F_1 15 chicks were obtained, all polydactylous. In F_2 out of 128 birds, 92 were polydactylous and 36 were normal. In a backcross to the Buttercup (normal) father there were obtained 20 polydactylous and 20 normal birds.

Bateson's White Leghorn $\times$ White Dorking material (quoted by Punnett and Pease, p. 348 and Table I) and some Silky crosses (Table II) gave comparable results.

(2) Abnormal behavior, with polydactyly incompletely dominant. Dorking $\times$ Brown Leghorn, and some of the Silky $\times$ Brown Leghorn crosses fall in this class. Records are given of F_1 's and an F_2 of Dorking $\times$ Brown Leghorn (Punnett and Pease, Table I) and of F_1 's, F_2 's, and backcrosses to both parents, of Silky $\times$ Brown Leghorn and Recessive White (a type extracted from a Brown Leghorn cross) (Punnett and Pease, Table II).

TABLE I

FREQUENCY ARRAYS OF PERCENTAGE OF NORMAL BIRDS IN BROWN LEGHORN AND RECESSIVE WHITE CROSSES. REARRANGED FROM PUNNETT AND PEASE

Per cent. normals	4 9 14 19 24 29 34 39 44 49 54 59 64 69 74 79 84 89 94 99																				Total
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	
Brown Leghorn F_1			2	1																	3
$\times$																					
Dorking F_2							1														1
(Brown Leghorn) F_1	4	1	4		1	1															11
and																					
(Recessive White) F_2						5	1	2	2	1	1		1						1		14
$\times$																					
Silky																					
(F_1 E.T.) $\times$ Normal									1		1	1	2	1	2	2	3				13
(F_1 n.e.) $\times$ Normal											1				1		2	2			6
$F_1 \times$ Silky	5		2					1													8

To the true F_1 's have been added families S60, S64 and S65. The polydactylous parents of these families were from a backcross of (Silky $\times$ Brown Leghorn) $\times$ Silky, and since they gave

less than 25 per cent. of normals when crossed with normals, they are assumed to be homozygous for the E.T. factor, but to lack some of the dominance factors. F_1 's then give from 0 to 26 per cent. of normal birds.

Nineteen backcrosses of heterozygous E.T. to normal birds were recorded, in which the heterozygote was either an extracted polydactylous bird (13 families) or an extracted normal bird which carried the E.T. factor (6 families). These gave from 40 to 86 per cent. of normal birds, and the families of which the heterozygous parent was normal gave on the whole a much higher proportion of normals than the rest.

Eight backcrosses of heterozygous E.T. to homozygous E.T. were recorded and gave from 0 to 35 per cent. of normal birds. In one case the heterozygote was normal in appearance, but none of its progeny was normal.

Fifteen F_2 families were recorded, and gave from 25 to 90 per cent. of normal birds.

Two heterozygous E.T. cocks from White Dorking crosses were bred both to Brown Leghorn hens and to hens of other breeds.

TABLE II

White Dorking $\times$ Indian Game			(White Leghorn $\times$ White Dorking) $\times$ (Indian Game $\times$ Brown Leghorn)		
Normal parent	Expt. No.	Per cent. normal progeny	Normal parent	Expt. No.	Per cent. normal progeny
Brown Leghorn	164	70	Brown Leghorn	168	61
White Leghorn 9	163	69	White Leghorn 565	167	40
do. 483	165	51	Buff Leghorn	169	52
do. 548	166	61	O. E. Game	170	50

Omitting the progeny of White Leghorn 9 (Expt. 163), breeds other than Brown Leghorn gave approximately 50 per cent. of normal birds, as expected when polydactyly is completely dominant, and Brown Leghorn gave considerably more than 50 per cent. of normal birds, as expected when some dominance factors are lacking. White Leghorn 9 was the only bird tested of its breed which seems to have lacked dominance factors.

(3) Normal Mendelian behavior with the four-toed condition dominant. Since all the polydactylous breeds which have been investigated gave a large proportion of polydactylous birds in F_1 , when crossed with normal birds, families in which normal behaves as a dominant will be expected only very rarely unless dominance factors are eliminated by selection.

Hen 461 from Expt. 40 (Silky $\times$ Brown Leghorn) was a normal heterozygote which must have lacked most of the dominance factors. She was used in Expts. S20, S43 and S58 (Punnett and Pease, Table II). In Expt. S58 she was mated with a normal Recessive White cock, and in a family of 14, only 2 birds were polydactylous. In Expt. S43 she was mated with a brother with an extra toe on one foot only, and in a family of 10 only one bird was polydactylous. In Expt. S20 she was mated to an F_1 cock from a Silky $\times$ Brown Leghorn cross in which all F_1 birds were polydactylous, and which therefore presumably carried dominance factors. In a family of 49 there were 14 normal birds, or 29 per cent.

In Davenport's Houdan experiments (quoted by Punnett and Pease, Table III) there were several crosses between heterozygous normal birds, and among these 86×83 (Table III B) gave 12 normal: 4 polydactylous; and 2324×936 (Table III B) gave 30 normals: 8 polydactylous, both very close to expectation in an F_2 with normal dominant.

Punnett and Pease classified their birds into "non-resistant" and "resistant" according to whether they gave progeny agreeing with Mendelian expectation (with polydactyly dominant) or differing from it. The assumption made in the present note is that such "resistance" factors affect only the heterozygote.

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INTERGENERIC HYBRIDS BETWEEN GOSSYPIMUM
AND THURBERIA

THE genera *Thurberia* and *Gossypium* are referred by Bentham and Hooker to the same tribe (Hibisceae) of the natural order Malvaceae. The chromosome number of the so-called Arizona wild cotton, *Thurberia thespesioides* A. Gray, has been determined by Dr. A. E. Longley (personal communication) as 13. The correctness of this number, which is the same as that of the cultivated Asiatic cottons, *G. stocksii*, and the wild Mexican cotton, *G. Davidsonii*, has been verified in this laboratory by J. E. Lans in acetocarmine preparations.

Thurberia thespesioides is morphologically very similar to *Gossypium*, and indeed was described by J. Miers (*Jour. Bot.*, xxxl, 330) as *G. lanceoeforme*.

Seeds of this species were obtained from Arizona through the kindness of Dr. T. Kearney and the resulting plants proved to be moderately well adapted to Trinidad conditions.

Attempts have been made to hybridize *Thurberia* with other species of *Gossypium*, and success has been attained in three instances.

(1) GOSSYPIMUM STOCKSII ♀ × THURBERIA THESPESIOIDES ♂

This cross was quite easily made, though the resulting plants were very weak and died just after the seedling stage after two or three leaves had been formed. The former species has deeply lacinated, while the latter has shallowly indented leaves. The hybrid possessed distinctly lacinated leaves, though it was not possible to state whether they were as lacinated as in *Thurberia*.

(2) GOSSYPIMUM DAVIDSONII ♀ × THURBERIA THESPESIOIDES ♂

Nine seedlings were obtained from three bolls. Brown glands appeared on the cotyledons and the latter rapidly developed an extensive necrosis leading to the death of all the seedlings before the second leaf was formed.

(3) NEW WORLD CULTIVATED (UPLAND × SEA ISLAND × SEA ISLAND) ♀ × THURBERIA THESPESIOIDES

One healthy plant of this cross was raised. The ♀ parent was Yy Pp Ss in composition (yellow corolla, yellow pollen, spotted) and *Thurberia* possesses a pale cream corolla, pale cream pollen and a petal spot. The hybrid plant was phenotypically yps,

which can be accounted for by assuming that the particular egg cell fertilized was yps. We should expect, however, that the hybrid would show dominance of spot from the *Thurberia* parent. That it does not may be provisionally explained if the ♀ parent carries two recessive spot genes and not one, since there is every reason to believe that there are, in New World cottons, two separate loci for S in different chromosomes. The present case would then be analogous to Bridges¹ triploid *Drosophilas*, in which he found that the combination of two recessive genes with one dominant was sometimes phenotypically recessive.

A comparison of the main features of the *Thurberia* hybrid and its parents is placed below:

	NEW WORLD	THURBERIA	HYBRID
Leaf shape	Moderately laciniated	Deeply laciniated	Intermediate
Leaf lobes	Predominantly 5	3	4-5
Bract teeth	Present (10-14)	Absent	Present (5-8)
Internal boll hairs	Absent	Present	Present
Bract size	Large	Small	Small
Bract (external glands)	Absent	Present (3)	Present (3)
Boll size	Large	Small	Intermediate (nearer to <i>Thurberia</i>)

Another successful cross has been made using as the female parent a plant of the composition Upland × Sea Island × Sea Island × Sea Island. Seven plants were raised, closely resembling the hybrid described above, except that they had yellow corolla, yellow pollen and a petal spot. Attempts to cross pure Peruvian, pure Upland or cultivated Asiatics by *Thurberia* have so far been without success.

Sterility of hybrids. The pollen of the hybrids has proved non-functional when applied to a wide range of types, including Peruvian, Upland, *G. Davidsonii*, *G. tomentosum*, *Thurberia*, *G. stocksii* and cultivated Asiatics. When selfed or when pollinated by pollen of the above forms, the capsules of the hybrid remain on the plant for a considerable period, but when mature show only shrivelled ovules devoid of embryos.

¹ Morgan, T. H., Bridges, C. B., and Sturtevant, A. H., "The Genetics of *Drosophila*," *Bibliographia Genetica*, Vol. II, 1925.

Taxonomic position of Thurberia thespesioides. A. Gray. Since *Thurberia* has 13 chromosomes and will cross with (a) an Asiatic species of *Gossypium* (*G. stocksii*) with 13 chromosomes, (b) a New World species (*G. Davidsonii*) with 13 chromosomes, and (c) cultivated New World cottons with 26 chromosomes, there seems to be no valid reason for excluding it from the genus *Gossypium*. We therefore propose to reinstate it in the genus under the name, *Gossypium lanceoforme* Miers.

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THE SPECTRUM ANALYSIS OF EYE COLOR IN *DROSOPHILA*

EYE color changes produced in *Drosophila* by gene mutation have been studied extensively. The comparison of eye colors has been made, however, by visual examination of the integrated spectrum of light reflected from the eye. This makes the description of an eye color difficult and a quantitative comparison between eye colors virtually impossible.

It seems that the problem might be considerably simplified by examining the wave-length distribution of the reflected light. Then the comparison of two eye colors would be reduced to the comparison of their wave-length distribution curves. An obvious application would be to the study of such allelomorphic series as that at the locus of white on the X-chromosome of *Drosophila melanogaster*.

Certain difficulties at once suggest themselves: the small amount of reflected light attainable from so small an object, the deterioration in the eye during exposure to intense light and the necessity of finding a method sensitive enough to show the small differences which exist between certain eye colors. The present investigation is of a preliminary nature and was undertaken to ascertain the possibility of overcoming the difficulties mentioned above.

The general method adopted was that of photographing the spectrum and of plotting the density curve for the photograph from microphotometer measurements. The eye to be examined

was severed from the head, pasted upon a piece of black paper in such a way as to prevent evaporation from the cut surface, and mounted on the stage of a compound microscope. Light from the hot pole of a carbon arc was passed through filters of water and blue glass and focused on the eye by means of a concave mirror. The eye was magnified 150x and focused on the slit of a large-dispersion spectrograph. Panchromatic plates were used to permit the study of the spectrum from 4000 to 8000Å. It was found that an exposure of from one to three minutes was ample and that no appreciable deterioration of the eye occurred in this time.

In this investigation two eye colors were compared, Red and Dilute-red. Dilute is a *dominant* eye color mutation obtained by radium irradiation. It has the apparent effect of slightly diluting red, vermillion and other eye colors. A more detailed account of its genetic behavior will be published later.

Some typical results are shown graphically in Figs. 1 and 2. The irregular shape common to all the curves is due to the varia-

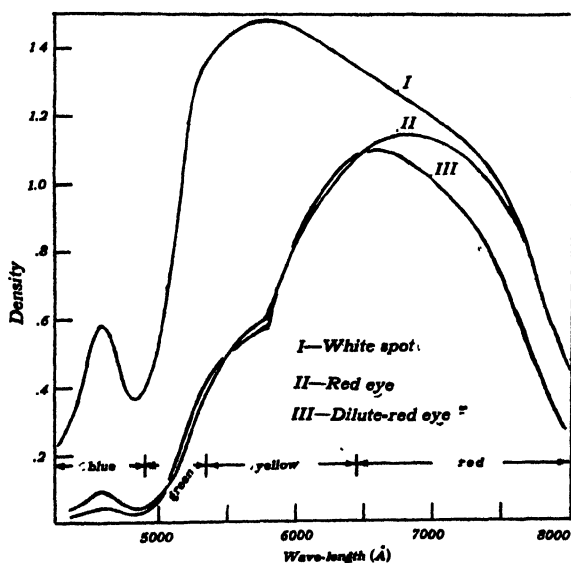


FIG. 1. Complete density curves (1 minute exposure).

tion with wave-length of the sensitivity of the photographic plates. Curve I is from a spectrum obtained by replacing the eye by a spot of white paper, and may be used as a standard for comparison. As would be expected, both the Red and Dilute-red eyes show a strong selective reflection in the red, with considerable reduction at the short wave-lengths. A comparison of curves

II and III shows significant differences in the red and blue portions of the spectrum, the Dilute-red eye showing less reflection in these regions. Curves IV and V of Fig. 2 were obtained with

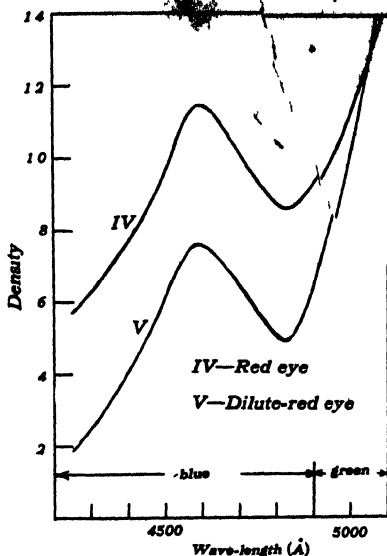


Fig. 2. Partial density curves (3 minute exposure).

longer exposure and bring out more clearly the difference at the shorter wave-lengths. The conclusion is that the effect of Dilute-red on the Red eye is a reduction of reflected light in two portions of the spectrum, the red and the blue.

This result is explained by histological examination of longitudinal sections of the ommatidia. The normal Red eye contains two kinds of pigment granules, purple-red and ocher-yellow. In the Dilute-red eye, the purple-red granules are found to be almost entirely absent from the distal portions of the ommatidia, and greatly reduced in number elsewhere. This would account for the loss in intensity in the red and blue spectral regions.

In order that results obtained in different experiments may be comparable, it is necessary to make allowance for the unequal energy distribution in the spectrum of the light source and for the variation of the sensitivity of the photographic plates with wave-length. This preliminary investigation has been sufficient, however, to show that the difficulties in the way of a quantitative study of eye color differences in *Drosophila* are not serious.

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TRANSPLANTATION OF TISSUES IN HYBRIDS OF INBRED FAMILIES OF GUINEA PIGS AND THE INDIVIDUALITY DIFFERENTIAL¹

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IN a former paper Loeb and Wright² have analyzed individuality differentials by means of transplantations within inbred families of guinea pigs. They also carried out transplantations between brothers and sisters within inbred families and compared the results thus obtained with those after transplantation from one inbred family to another and after ordinary homoio, syngenesio and autotransplantation. In addition they studied the effect of transplanting from a hybrid between two inbred families to one of the parent families or *vice versa* from one of the parent families to a hybrid. The results obtained in the latter experiments made it possible to define the conditions under which reciprocal transplantations may yield different results, and furthermore indicated the cause of the reactions of the host against the transplant.

According to the theory of the individuality differential³ we should expect that if we carry out transplanta-

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² Leo Loeb and Sewall Wright, *Amer. J. Path.*, 1927, 3, 251.

³ Leo Loeb, *AM. NATURALIST*, 1920, 54, pp. 45 and 55; *Physiol. Reviews*, 1930, 10, 547.

tions from hybrids between two inbred strains to other hybrids between the same strains or to hybrids in which only one of the component strains is the same while the other differs, the results are less favorable than if we transplant tissues within the same strain, because in the former case the chances that the genetic constitution of the donor tissue differs from that of the host tissues is greater than in the latter cases. During the last two years we undertook a series of such transplantations with a view of testing this supposition. While the number of animals which was at our disposal for this purpose was unfortunately not very large, we believe that, notwithstanding this deficiency, the results obtained are definite in several respects.

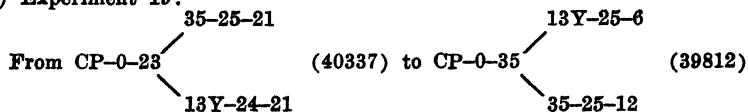
In the paper of Loeb and Wright there were recorded only two experiments in which tissues were exchanged between hybrids composed of the same two parent strains. In one of those two experiments a hybrid between strains 2 and 35 was used. Examination took place after 31 days and the result was 5, corresponding to syngenesiotransplantation. In the second experiment a hybrid between strains 35 and 32 was used, examination took place after 35 days and the result was 6, corresponding to autotransplantation.

In our present series two kinds of hybrids were at our disposal: (1) Hybrids between families 35 and 13, designated as CP; and (2) hybrids between families 13 and 2, designated as CY. In CY-0, a line of family 13, rich in individuals with otocephalic defects, was used and in CP-0 the same line of family 13 was likewise used in many cases. CP-0 and CY-0 represent the F_1 hybrids between these families. CP-1 and CY-1 designate the first generation of brother-sister matings from experiment CP-0 and CY-0, respectively, while CP-2 and CY-2 represent the corresponding second generations of brother-sister matings.

We shall give a list of the experiments of this kind carried out and of the results obtained. While as a rule we

shall state the results briefly, in a few cases we shall describe them in somewhat more detail.

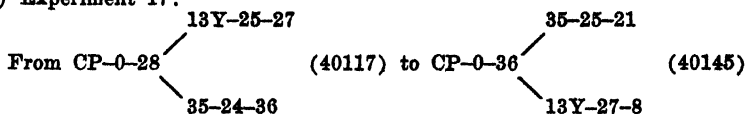
(1) Experiment 19:



Examination after 20 days. Results approach but do not quite equal those of autotransplantation. The thyroid transplant resembles, as far as its structure is concerned, an autotransplant, except that in some places there is near the border of the inner ring of the transplant a certain amount of dense fibrous tissue. The ring of acini is well preserved; the acini and the colloid they contain are normal and usually the acini are close together. However, the number of lymphocytes is slightly increased; they fill loosely some of the small lymph vessels. The fat tissue around the thyroid is, on the whole, well preserved, but there is in places a little too much connective tissue growing into it and there is also here and there a small increase in lymphocytes. We find, furthermore, a new formation of epithelioid cells. While the parathyroid is on the whole well preserved, one part of this structure has been almost entirely replaced by masses of lymphocytes, still enclosing remnants of parathyroid tissue and a number of lymphocytes are seen infiltrating the neighboring parathyroid. The cartilage is well preserved although some parts of it are necrotic probably owing to the injury received during the process of transplantation; there are in places small collections of lymphocytes in the connective tissue surrounding it. The fat tissue around the cartilage is also well preserved and contains few lymphocytes. The transplanted muscle fibers show normal cross striation and nuclear chains as an indication of regenerative processes which have taken place. The surrounding connective tissue shows an increase in the number of lymphocytes. The transplanted bone is in part surrounded by osteoblastic tissue and it is possible that some light staining areas of bone are

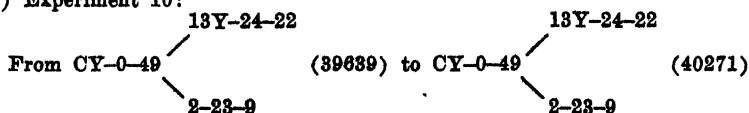
newly formed. The bone marrow is replaced by large capillaries and some loose connective tissue. There are probably some bone marrow cells (myelocytes) left.

(2) Experiment 17:



Examination after 41 days. The result approaches that of homoiotransplantation. Instead of thyroid transplant, only fibrous tissue is found. The cartilage transplant is mostly preserved, including the columns of cartilage cells near the bone; however, a thin zone of necrotic cartilage is in process of calcification. The cartilage is surrounded by fibrous tissue. Where the cartilage is necrotic, connective tissue penetrates into it. Also a mass of lymphocytes pushes its way into the center of the cartilage, where it is apparently undergoing calcification; furthermore, masses of lymphocytes enter the cartilage-bone border. In addition there is some lymphocytic infiltration in the connective tissue around the cartilage. Here the connective tissue is finer in structure than is found in typical cases of homoiotransplantation. In this specimen the reaction of the host tissue against the transplant seems to be less violent. The bone is necrotic and the bone marrow is replaced by loose fibrillar connective tissue. Around the bone there is fibrous tissue with lymphocytic infiltration of medium intensity. Near the cartilage-bone border the fat tissue contains epithelioid and giant cells.

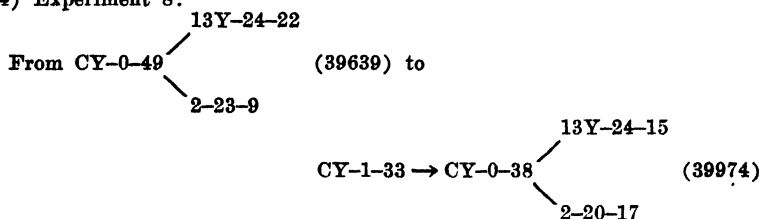
(3) Experiment 10:



Examination after 21 days. Host and donor have in this case the same genetic constitution, although they are not brothers. In accordance with expectation the result corresponds to that of autotransplantation. Thyroid,

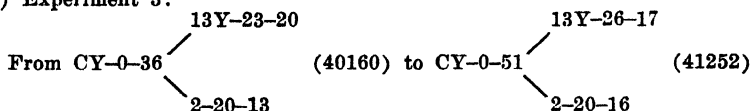
parathyroid, cartilage, bone, muscle and fat tissue behave as they do in case of autotransplantation. The transplant as a whole is very well preserved, and also the bone marrow is at least partly preserved, while another part of it is necrotic. Near the cut end it is replaced by loose connective tissue which has penetrated into it from the outside. The number of lymphocytes does not exceed that seen in autotransplants.

(4) Experiment 8:



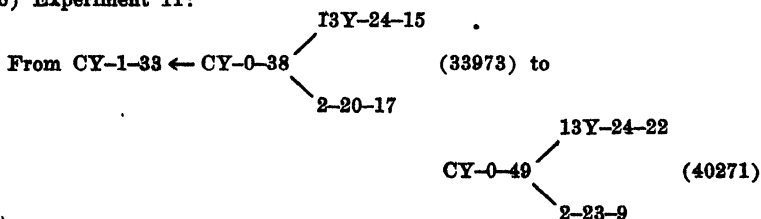
Examination after 21 days. The constitution of host and donor are not identical, but similar. Results correspond about to autotransplantation.

(5) Experiment 3:



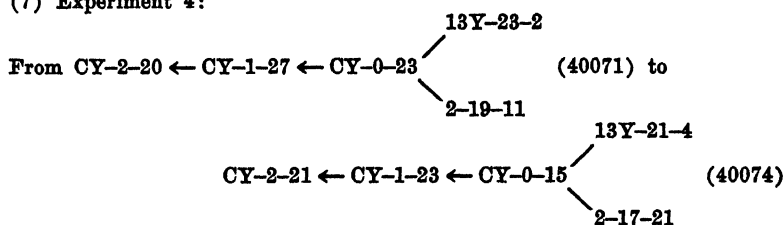
Examination after 25 days. Constitution as far as family 2 is concerned is very similar; there is, however, a slight difference in regard to family 13. In this case thyroid and parathyroid behave like autotransplants; the necrosis of great parts of the transplanted cartilage may be due to accidental conditions. We find in the bone marrow, which is partly preserved, some newly formed osteoid tissue as the result of the activity of osteoblastic tissue.

(6) Experiment 11:



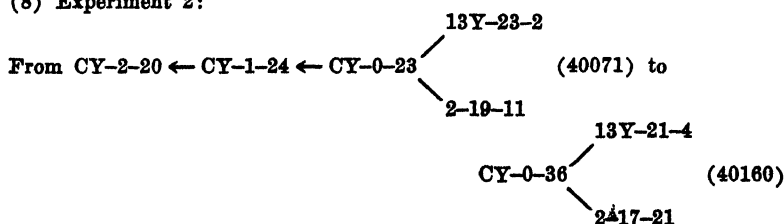
Examination after 21 days. Constitution of family 13 in these hybrids is very similar, but there is some difference in the constitution of family 2. As far as preservation and structure of thyroid, cartilage and bone marrow are concerned, they correspond about to autotransplantation; however, there is some increased lymphocytic infiltration in thyroid and around cartilage and some increase in fibrous tissue in various places including the fat tissue. The results correspond to those seen in favorable cases of syngenesiotransplantation.

(7) Experiment 4:



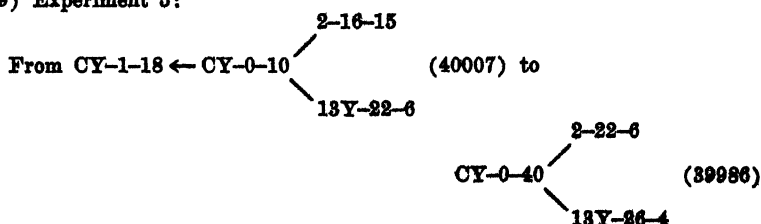
Examination after 25 days. In this case constitution of family 13, as well as of family 2, differs somewhat in both hybrids. Homoioreaction.

(8) Experiment 2:



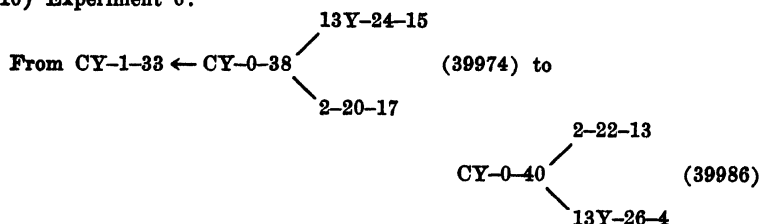
Examination after 25 days. The hybrids differ in constitution of family 13 as well as of family 2. On the whole, homoioreaction.

(9) Experiment 5:



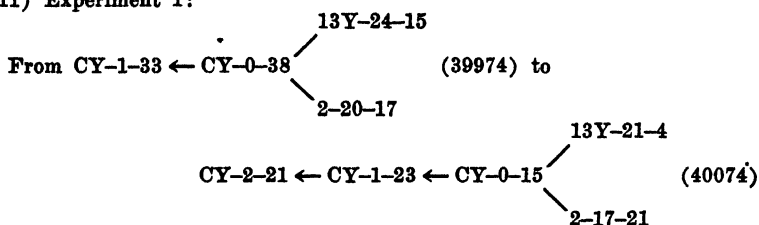
Examination after 20 days. Homoioreaction. The hybrids differ in the constitution of family 13 as well as of family 2.

(10) Experiment 6:



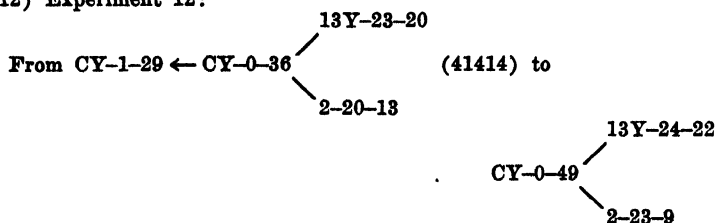
Examination after 20 days. Homoioreaction. Both hybrids differ in constitution of family 13 as well as of family 2.

(11) Experiment 1:



Examination after 20 days. Homoioreaction. Family 13 as well as family 2, entering these hybrids, differ somewhat in constitution.

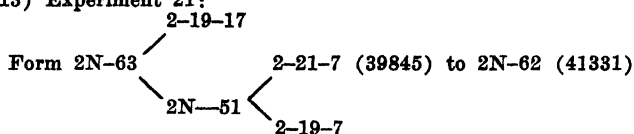
(12) Experiment 12:



In this case the uterus had been transplanted. Examination after 10 days. Surface epithelium, glands as well as muscle tissue are fairly well preserved; but parts of transplant are necrotic. There are some small collections of lymphocytes. The reaction was unlike that found in autotransplantation.

In addition, we carried out two experiments in which instead of hybrids between 2 families, hybrids between different branches within the same family were used for transplantation. We have therefore in these cases not to deal with as closely inbred individuals as in the transplantations within the same strain used formerly by Loeb and Wright, where there had been consistent brother and sister mating. The animals designated "2N" were selected from family 2 for colored nose spots.

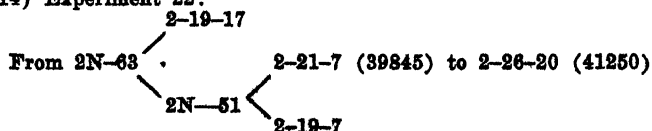
(13) Experiment 21:



2N-62 has a very complicated pedigree. It comes through 9 generations of selection on the sire's side for pigmented nose spots (non-brother-sister matings) to the strict brother and sister matings in inbred family 2. The sire's sire and dam, however, are brother and sister from the selection experiment. On the dam's side there are 3 generations of non-brother-sister matings till we reach the continued brother-sister matings in family 2.

Examination after 25 days. Thyroid is only partly preserved. In the majority of the acini no colloid is left. While there is only a very slight lymphocytic reaction, there is an increased amount of connective tissue around some acini. Cartilage is on the whole well preserved. In the fat tissue there is in places a slight connective tissue ingrowth and a slight epithelioid reaction. There is a very moderate lymphocytic infiltration around the cartilage and also in the fat tissue there are some lymphocytes. Bone marrow is not preserved. We have to deal here with a syngenesio, approximately intermediate between an autogenous and a homogenous reaction.

(14) Experiment 22:



Examination after 31 days. In this case the host had been inbred through 26 generations of brother and sister matings. Thyroid, cartilage, bone and bone marrow are relatively well preserved, but there is a slight increase in lymphocytes and a very moderate increase in connective tissue. We have therefore to deal with a less severe syngenesio reaction than that obtained in the preceding Experiment 21.

DISCUSSION

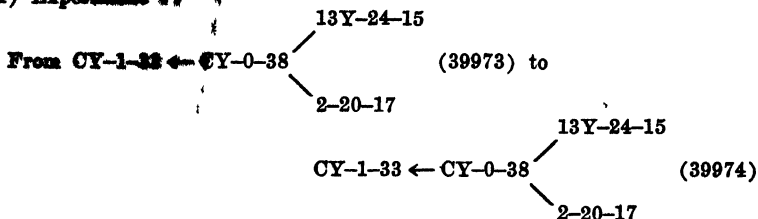
In this series we have to deal with 12 experiments in which tissues were exchanged between hybrids in the constitution of which two different inbred families entered: but in each experiment the donor and host were offspring from the same parent strains. In two additional experiments host and donor belonged to the same family, but either host and donor were not the result of strict brother and sister matings, but were for several generations hybrids between individuals other than brother and sister of family 2, or the donor was such a hybrid, while the host was closely inbred.

The first 12 experiments can be divided into two classes: a first one (a) consisting of five experiments in which the results approached or even reached the conditions characteristic of autotransplantation, including one case with a result corresponding to a favorable syngenesiotransplantation, and a second one (b) consisting of six experiments in which the results approached or reached those characteristic of homoiotransplantation. There was one experiment (12), in which, although the examination took place too early to allow of a definite grading of the result, we can be certain that it did not correspond to the condition characteristic of autotransplantation. In class (a) each of the two families, which entered into the constitution of donor and host, belonged to the same generation of brother and sister mating

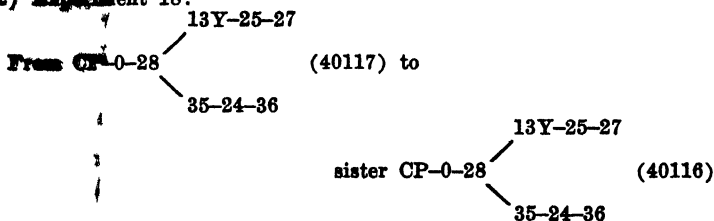
within that particular family, while in (b) this held good only in the case of one of the families entering the constitution of this hybrid, while in the case of the other family the generations differed. This sharp division between the behavior of these two kinds of hybrids may be a coincidence, if we consider the results as a whole, but in individual cases the more favorable outcome of the transplantation may be due to a closer relationship existing between the donor and the host in class (a) than in class (b).

In the last two experiments (13 and 14), the results were inferior to the average results obtained by Loeb and Wright in transplantations within closely inbred families. Hybridization within the same family may be expected to lead to a slightly less homogeneous genetic condition in donor and in host than in case donor and host have been propagated strictly by brother and sister matings within the same family. Greater still are the chances for a difference in constitution in the first 12 experiments. Where two different families enter the constitution of each family, there is a greater probability that the genetic constitution of donor and host are dissimilar than when both father and mother belonged to the same inbred family. Correspondingly the results obtained in these transplantations are much less favorable than the results obtained by Loeb and Wright in transplantations within the same inbred family. In the latter experiments the results varied between those characteristic of auto and syngenesiotransplantation. In the new experiments in about one half of the cases results were obtained approaching those found in homoiotransplantation. While in the earlier transplantations the average results were definitely superior to those noted in brother to brother transplantations in ordinary, non-inbred stock of guinea pigs, in this series they are very similar or only very little superior to those obtained in brother to brother matings in non-inbred stock.

**TRANSPLANTATION FROM BROTHER TO BROTHER (OR SISTER),
WHOSE FATHER AND MOTHER BELONG TO
DIFFERENT INBRED FAMILIES**

(1) Experiment 9:

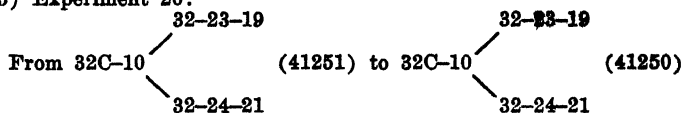
Examination after 21 days. In this case transplanted cartilage, fat and muscle tissue approximated in their condition autotransplants; they were on the whole well preserved. Only very few lymphocytes were seen and there was hardly any increase in connective tissue. Cartilage and muscle tissue showed areas of regeneration. The regeneration of cartilage as usual was observed either in places where the tissue had been wounded or where preserved perichondrium surrounded necrotic cartilage.

(2) Experiment 18:

Examination after 41 days. Thyroid and surrounding fat tissue on the whole showed the character of autotransplants; but there were small collections of lymphocytes in various places. The same condition was found in the case of cartilage, bone, bone marrow and muscle transplants. There was in places a slight increase in connective tissue and there were some small collections of lymphocytes. The transplants approached, therefore, but did not quite reach the condition found in autotransplantation.

In addition, in two cases brother to brother transplantations between hybrids within the same family were made. The guinea pigs represented the F_1 generations of a mating between two guinea pigs both belonging to family 32, the parents not being brother and sister.

(3) Experiment 26:



The animals are brothers. Examination after 25 days. Thyroid, cartilage, fat and muscle tissue behaved like autotransplants.

(4) Experiment 27. The reciprocal transplantation to preceding experiment (41250 to 41251). Examination after 25 days. Tissues behaved like autotransplants.

DISCUSSION

While these experiments are few in number they are in harmony with the previous results of Loeb and Wright. In accordance with our earlier observations we find that the tissues behave like autotransplants, if brother and sister belong to the same inbred family; this holds good even if father and mother were not brother and sister, but belonged to the same inbred strain. However, we may expect that if a very large series of transplantations of this kind were compared with brother and sister transplantations in strictly inbred families, the average result in the former series would not be quite so good as in the latter. With our small number of experiments this result is not manifest. However, if brother and sister represent hybrids between two different inbred families, instead of belonging to the same closely inbred family, the results, although still very good and approaching those of autotransplantation, are distinctly poorer than in the case of brother and sister transplantations within the same closely inbred family.

If we combine our present results with the previous ones of Loeb and Wright, we obtain the following re-

sults: In case of transplantation within the same inbred family (including two F_1 hybrid transplantations within the same inbred family) we obtained in 14 out of 16 transplantations auto reactions, in one case the result approached an auto reaction, and in the last case a syngenesio reaction was obtained. We carried out altogether 10 transplantations in which brothers or sisters were hybrids between two different inbred families. In 6 of these cases the results corresponded to those obtained in autotransplantation, while in 3 cases the result approached but did not reach that of autotransplantation and in one case a syngenesio reaction was found. In accordance with theoretical expectations, we find then that hybridization, in increasing the probability of a difference in the genetic composition of host and donor, makes the result of brother and sister transplantations somewhat less favorable. However, if we compare the results of the latter type of brother and sister transplantations with the ordinary kind of brother and sister transplantations, as carried out by Loeb⁴ and by Loeb and Wright,¹ the result in the latter experiments is much inferior, as might be expected if we consider that in ordinary brother to brother (or sister) transplantations the chances that the genetic composition of two brothers is the same are much poorer than in cases of transplantation within the same inbred families where the genetic constitution is more homogeneous.

HOMOIOTRANSPLANTATIONS

In two cases we carried out transplantations between animals which genetically were not related to each other, although in one of these two cases in which a B animal was used as the host, host and donor ultimately were derived from the same stock. The B group, a considerable number of years ago, originated from the same stock as the inbred families, but, in breeding the B group,

⁴ Leo Loeb, *J. Med. Research*, 1918, 38, 393; 39, 39, and 1920, 41, 305; *Am. J. Path.*, 1927, 3, 29 and 45.

matings as close as those between second cousins have been avoided in contrast to the close inbreeding practiced in the case of the inbred families.

(1) Experiment 9:

13Y-21-4
 From CY-1-27 ← CY-0-18 (40040) to 13Y-21-4 (40100)
 2-18-7

Examination after 21 days. Of the thyroid transplant only dense hyaline fibrous tissue with some lymphocytes remains. The fat tissue shows epithelioid reaction. Around the cartilage which is partly preserved and in which some regenerated areas are seen, there is much lymphocytic infiltration, especially around the places of regeneration, and lymphocytes penetrate even into the newly formed, still cellular cartilage. A great part of the fat tissue has been replaced by fibrillar connective tissue. In the remaining fat tissue there is some epithelioid reaction. Bone marrow is replaced by fibrillar connective tissue in which there is some lymphocytic infiltration. The transplanted muscle tissue is necrotic. The result of transplantation corresponds therefore to that to be expected after homoiotransplantation.

(2) Experiment 20:

35-25-21
 From CP-23 to one of our stock guinea pigs.
 13Y-24-21

Examination after 20 days. In this experiment the results are very similar to those obtained in the preceding experiment. However, there was still a mass of thyroid tissue preserved in this case. The contrast between the results obtained in these homoiotransplantations and in the preceding syngenesiotransplantations is very great and both results are in accordance with expectations.⁵

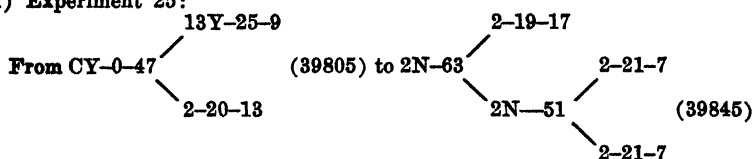
⁵ Leo Loeb, *Am. J. Path.*, 1926, Vol. 2, pages 111, 99, 801 and 812.

TRANSPLANTATION FROM HYBRID TO ONE OF THE COMPONENT FAMILIES AND VICE VERSA

In former experiments Loeb and Wright found that transplantations from a hybrid between two inbred families to one of the component inbred families gave an average grade of 3.25, signifying a decided reaction on the part of the host against the transplant. In the various experiments the results were as follows: In one case there was an auto reaction; in three cases favorable syngenesio reactions; in eight cases homoio reactions and in twelve cases reactions intermediate between homoio and syngenesio reactions were obtained. It was quite different in transplantations from one of the parent inbred families to a hybrid between itself and a different inbred family. In the latter type of transplantation the average grade was 5.77. In this series the results were as follows: In eight cases the grafts behaved like auto-transplants and in the six remaining experiments they behaved like favorable syngenesiotransplants. We can now add to the former experiments four additional ones from our new series.

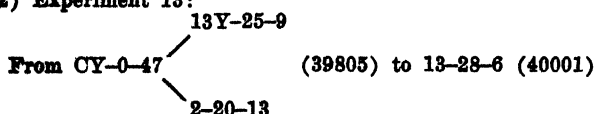
(a) TRANSPLANTATION FROM HYBRID TO ONE OF THE PARENT FAMILIES

(1) Experiment 25:



Examination after 22 days. A marked homoioreaction took place. No thyroid was preserved; there were strong lymphocytic and connective tissue reactions in the transplants.

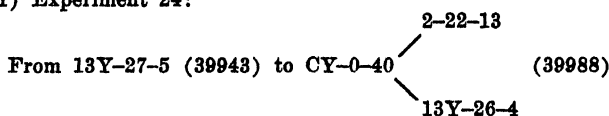
(2) Experiment 13:



In the transplant only very small parts of thyroid tissue were preserved, and there was a distinct lymphocytic reaction. The fat tissue showed epithelioid character; it was partly replaced by fibrous tissue and there was some infiltration with lymphocytes. The cartilage was surrounded by dense fibrous tissue with a moderate or, in places even a dense lymphocytic infiltration. While the result obtained in this experiment was somewhat more favorable than in the preceding experiment, it still came within the range of a homoioreaction or at least approached the latter.

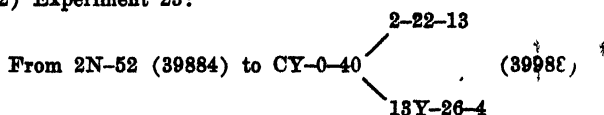
(b) TRANSPLANTATIONS FROM ONE OF THE PARENT FAMILIES TO HYBRID

(1) Experiment 24:



Examination after 31 days. Thyroid as well as cartilage transplant was very well preserved. Both resemble autotransplants except that in one place there is a definite lymphocytic infiltration in the thyroid. While the result in this experiment is in agreement with our former conclusions, the second one behaves in an exceptional way.

(2) Experiment 23:

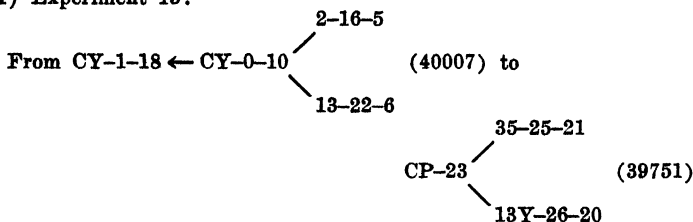


Examination after 31 days. No thyroid was found and the cartilage behaved like a homoiotransplant. Whether in this experiment, which apparently differed in its results from all others, a mistake occurred in the identification of the specimens, or whether the unfavorable outcome is due to the constitution of 2N-52 which represents a very complex 2N hybrid, we must leave undecided.

**TRANSPLANTATION OF TISSUES IN HYBRIDS, IN WHICH ONE
PARENT FAMILY IS THE SAME IN DONOR AND HOST,
WHILE THE OTHER PARENT FAMILY
DIFFERS IN BOTH**

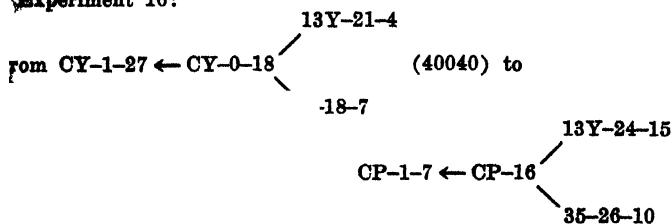
We carried out three transplantations of this kind.

(1) Experiment 15:



Examination after 20 days. A ring of thyroid tissue is preserved; many acini still contain colloid, others are without colloid. In places connective tissue bands separate acini from each other. The connective tissue in the center of the transplant is increased. There is much lymphocytic infiltration; also the parathyroid is partly replaced by collections of lymphocytes. Cartilage is preserved, but surrounded mainly by fibrous tissue. Fat tissue is still partly preserved, but is much infiltrated with lymphocytes. Transplanted muscle tissue is well preserved; but again there is much lymphocytic infiltration in the connective tissue separating the muscle fibers. Bone marrow is replaced by connective tissue, which in part is highly fibrillar. The transplant is intermediate between a syngenesio- and homoio-transplant.

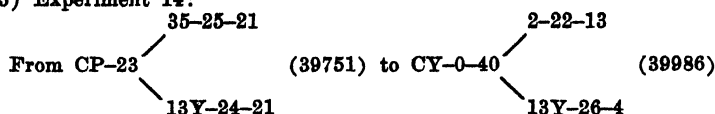
Experiment 16:



Examination after 21 days. Here thyroid, cartilage, bone, bone marrow, muscle and fat tissue are relatively

well preserved; but there is a distinct increase in lymphocytes in various places and at certain points there is also an increase in connective tissue. The result corresponds to syngenesiotransplantation.

(3) Experiment 14:



Examination after 19 days. Thyroid has been destroyed and replaced by fibrous tissue and some collections of lymphocytes. Cartilage is surrounded by dense fibrous tissue and a dense mantle of lymphocytes. Bone marrow is largely replaced by connective tissue. Fat tissue is to a great extent infiltrated with epithelioid and giant cells. Homoioreaction.

In the last experiment in which a hybrid 2/13 is the host, the reaction is more pronounced than in the others; this result corresponds to analogous observations of Loeb and Wright, who likewise noticed that hybrids between families 13 and 2 react very actively against strange tissues. The number of these experiments is as yet too small for definite conclusions; but, as far as the results obtained so far go, they are of a similar kind to those found in transplantations from hybrid between two inbred families to one of the component parent families. Such a similarity in the reactions in these two series of experiments might be expected, because in both cases the host reacts against a strange set of genes present in the transplant, while another set of genes is similar or identical in both host and transplant. However, while in the transplantation from hybrid to one of the parent families, the host does not contain a set of genes which is not represented in the transplant, in the kind of transplantations which we are considering here, there is found in the host a set of genes which is strange to the donor. It is an interesting problem whether this admixture in the host alters the reaction to the transplant. In order to

decide this question it would be necessary to enlarge this series of transplantations considerably and to compare the results obtained with those in transplantation from hybrid to one of the component parent families.

DISCUSSION

The principal conclusions which may be drawn from our experiments have been discussed at the end of the different chapters dealing with the various kinds of experiments. We shall here emphasize some of the more general conclusions. In the first place it follows from our experiments definitely that in correspondence with the chance of greater genetic dissimilarities in case we have to deal with hybrids between different families as compared with animals belonging to the same closely inbred family, the results of transplantation are less favorable in the first than in the second case. It follows, furthermore, that the results are better if the families represented in the genetic constitution of the donor are also represented in the host than when the donor contains a genetic constituent which is not present in the host. Thus in transplantation of tissues in hybrids whose parents belonged to different families, but in which the families were the same in host and donor, in a considerable number of cases results approaching autotransplantation were obtained; this was not so, when one family present in the donor was not represented in the host.

Also the result of our brother to brother transplantations confirm these conclusions. In case the brothers are hybrids between the same two inbred families the results of transplantation are slightly less favorable than in case we have to deal with brothers within the same closely inbred family, but they are always better than those of ordinary brother to brother transplantation within non-inbred families. Homoiotransplantations used as controls behaved in the usual manner and no difference could be found between homoiotransplantation

between guinea pigs derived from entirely unrelated stock and between guinea pigs which many generations back had descended from the same stock.

Thus, on the whole, our results are confirmatory of the conclusion that reactions of a host against a transplant represent a finely graded indicator of the genetic relationship between individuals and can therefore be used in the analysis of individuality differentials.

SUMMARY AND CONCLUSIONS

(1) If instead of transplanting tissues from one member of an inbred family of guinea pigs to another member of the same family, we transplant from a hybrid between two inbred families to another hybrid of the same kind the results become more unfavorable in accordance with the greater difference in average genetic constitution between host and donor to be expected under these conditions. In our experiments the most favorable results were obtained in those cases in which in host and donor the families of father as well as of mother belonged to the same generation in both hybrids. In accordance with expectation the average of the results obtained in this series of transplantations, as a whole, is much superior to ordinary homoiotransplantations and corresponds approximately to the results of ordinary (brother to brother) syngenesiotransplantation. There is also an indication that exchange of tissues between hybrids within the same family will yield somewhat more unfavorable results than exchange of tissues between strictly inbred individuals within the same family.

(2) The result of transplantations between hybrids of the same inbred families, when donor and host are brothers or sisters, is much superior to ordinary syngenesiotransplantations in non-inbred families, but is somewhat less favorable than transplantations between non-hybrid brothers belonging to the same inbred family; these results are also in accordance with differences in the genetic constitution in such animals.

(3) In accordance with expectation, transplantation from hybrids between inbred families to non-related guinea pigs either non-inbred or representing hybrids between two inbred families other than those used for the first hybridization, give ordinary homoioreactions.

(4) In addition we carried out a small number of transplantations from one of the parent strains to a hybrid between two inbred families and vice versa from a hybrid to one of the component parent strains. The former type of transplantations yielded favorable results in a former large series of experiments of Loeb and Wright. One new experiment of this kind agreed with the former ones, while in a second case an abnormal result was obtained, presumably due to an accidental error. Transplantations from a hybrid to one of the parent strains yielded results varying between syngenesio and homio reactions in accordance with our previous results. Similar to the latter also were the results of transplantation between two hybrids in which one of the component parent families was the same, while the second parent family was different.

(5) In accordance with previous conclusions of Loeb and Wright, we may assume that the severity of the reaction against the transplant is determined by the number of strange gene-derivatives which are introduced with the transplant into the host and by the degree of their strangeness. A comparison between the results obtained in transplantations from hybrid to component parent strain on the one hand, and in transplantations between hybrids in which one family entering into the hybrid constitution is the same while the other family differs, on the other hand, if based on a large series of experiments, should answer the question whether in addition to the strangeness of the gene derivatives, introduced with the transplant into the host, the presence of strange gene derivatives in the host plays a rôle in determining the intensity of the reactions of the host against the transplant.

ON THE NATURE OF SIZE FACTORS IN MICE

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INTRODUCTION

THE nature of the factors controlling size in plants and animals has long been of interest to geneticists. In a few cases the difference between large and small size is apparently due chiefly to a single segregating gene. Such were Mendel's tall and short peas. Other examples are the "giant" mutation in *Drosophila melanogaster*, described by Bridges and Gabritschevsky (1928), and "dwarf" in mice reported by Snell (1929). More frequent are the examples in which the size of a particular part of the organism is determined by a single gene. Lindstrom (1926) found small fruit size in *Lycopersicum* partially dominant in some cases. Short-ear in mice behaves as a simple recessive to normal ear (Lynch, 1921). Another example is the "tailless" condition in mice (Lang, 1912). But still more numerous are the many instances in which size inheritance is "blending." This is the usual mode in both plants and animals.

It is now generally agreed that the genetic basis of size is prevailingly dependent on several or many genes. Davenport (1917) concluded that in humans the factors for tallness were mostly recessive. Usually, however, the multiple factors involved are without dominance and the inheritance is blending. The multiple factor hypothesis was employed by Phillips (1912, 1914) to explain the results of size inheritance experiments with ducks and by MacDowell (1914) for rabbits, Punnett and Bailey (1914) for fowls and Castle (1922) for rabbits.

Recently, however, Castle (1929) after further work on the inheritance of size (weight) in rabbits has stated that it is an open question whether general body size is determined by chromosomal genes. He was unable to detect any evidence for linkage between size and any of several

Mendelian qualitative characters affecting coat color. On the other hand, the author (1930, 1931) found indications of genetic linkage between qualitative and quantitative characters in a mouse species cross. This finding would tend to show that at least some of the size factors in mice are chromosomal.

A mooted question concerning the nature of size factors in mammals is whether they are general in their action, influencing all parts of the body as Castle (1924, 1930) believes them prevailingly to be, or whether they are specific, affecting primarily certain definite parts. Sumner (1924) believes that there are such local factors in *Peromyscus*, and Davenport (1930) holds the same for man. In the papers previously cited, the author stated his belief that in mice the differences in the behavior in inheritance of various quantitative characters suggested the existence of local as well as general factors for size. In the present paper it is proposed to examine statistically some of these differences in an endeavor to throw some light on the nature of size factors in mice.

MATERIAL

The material investigated in this study consists of several quantitative characters of a race of small Chinese mice, *Mus bactrianus* (the descendants of wild individuals brought from China in 1926), an inbred strain of dilute brown non-agouti *Mus musculus*, the F_1 hybrids, and a back-cross generation produced by mating *musculus* females to F_1 males. The quantitative characters considered are the following: body length, tail length, adult weight, skull length, skull width, humerus length, length of femur, transverse diameter of femur, sagittal diameter of femur, diametric ratio of femur, tibia length and cranial capacity. No mouse of a lesser age than 181 days was included in the computations, so it is probable that the size characters had attained their maximum development.

Body length and tail length were measured by a method similar to that described by Sumner (1927).

The length of the body by this method is the total length less tail length.

In a study of growth, the mice were weighed every tenth day from the thirty-first to the one hundred eighty-first day. The maximum value recorded for a mouse was considered its adult weight.

Skull length is the distance from the highest point of the foramen magnum to the most anterior surface of the incisor teeth.

Skull width is the inter-orbital width, the width of the frontal bones between the points of their strongest lateral constrictions. Because of the curvature of the frontal bones in the region adjacent to these points this dimension in the small skulled *bactrianus* could not be measured, by means of the Starrett bench micrometer employed for skeletal measurements, with as great accuracy as might be desired. Hence this measurement is considered only in the case of the larger skulled *musculus*.

Humerus length is the distance from the highest point of the head to the most distal point of the external side wall of the trochlea.

Femur length is taken as the distance from the highest point of the first trochanter to the deepest point of the internal condyle.

Transverse diameter is the least diameter, taken transversely, of the shaft of the femur between the third trochanter and the distal extremity.

Sagittal diameter is the diameter, taken antero-posteriorly, of the shaft of the femur at the level of the transverse diameter described above.

Diametric ratio of the femur equals the sagittal diameter divided by the transverse.

Tibia length is measured from the most proximal point of the articulation surface to the most distal point of the medial malleolus.

The humerus, femur and tibia measured were always the right.

The cranial capacity was determined by filling the skull with mercury, which was then weighed. Repeated measurements rarely showed deviations exceeding one half of one per cent. The approximate capacity in cubic centimeter may be obtained by dividing the values given by 13.5438 (the specific gravity of mercury at 21° C.).

CONSIDERATION OF DATA

Table I presents the mean values of eleven quantitative indices for *bactrianus*, *musculus*, and the first generation hybrids. It will be seen that the F_1 means fall into two general classes: one in which the value is intermediate between those of the parental species, and the other in which the F_1 mean equals or exceeds the mean of the larger parent race. In every instance the F_1 mean is significantly greater than that of the smaller parent. With the exception of the diametric ratio of the femur, the "larger parent" race is *musculus*.

The first class (those characters for which the deviation of the F_1 from the larger parent is greater than three times its probable error with the sign negative) includes the following: body length, tail length, adult weight, humerus length, transverse diameter of femur, sagittal diameter of femur (females) and diametric ratio of femur. The second class (those characters for which the deviation of the F_1 from the larger parent is positive in sign or less than three times its probable error with the sign negative) comprises the following: skull length, femur length, sagittal diameter (males), tibia length and cranial capacity. The F_1 males significantly exceed *musculus* males in skull length; femur length and sagittal diameter. This may be explained by the circumstances that the sex differences for these characters as well as for the diametric ratio are more marked in *musculus* than in *bactrianus* or in F_1 animals.

These two classes of F_1 means seem to show that not all factors influencing size which go into the hybrid animals from their diverse parents are general in their

TABLE I
MEAN VALUES OF QUANTITATIVE INDICES

	<i>bactrianus</i>			<i>musculus</i>			F_1 <i>musculus</i> ♀ × <i>bactrianus</i> ♂			Deviation of F_1 from larger parent	Deviation P.E.
	No.	Mean	No.	Mean	No.	Mean	No.	Mean	Mean		
Body length (mm)	♂ 17	80.2	± .51	72	95.8	± .23	39	90.8	± .40	- 5.0	± .46
	♀ 26	79.7	± .49	56	98.3	± .50	36	91.3	± .45	- 7.0	± .67
Tail length (mm)	♂ 12	68.8	± 1.22	63	93.7	± .24	32	85.7	± .37	- 8.0	± .44
	♀ 21	67.7	± .53	56	92.0	± .38	32	85.0	± .44	- 7.0	± .58
Adult weight (gms)	♂ 19	15.5	± .17	35	31.3	± .47	37	22.0	± .32	- 9.3	± .57
	♀ 14	12.8	± .23	32	30.9	± .76	16	18.3	± .36	-12.6	± .84
Skull length (mm)	♂ 35	20.40	± .050	79	21.67	± .032	39	21.98	± .040	+ .31	± .051
	♀ 33	20.52	± .058	93	21.89	± .043	37	22.08	± .059	+ .19	± .073
Humerus length (mm)	♂ 19	10.44	± .054	67	11.37	± .020	39	11.25	± .029	+ .12	± .035
	♀ 29	10.23	± .044	90	11.30	± .026	40	11.00	± .034	- .30	± 0.43
Feet:											
Length (mm)	♂ 37	13.76	± .058	70	14.26	± .033	39	14.67	± .044	+ .41	± .055
	♀ 37	13.74	± .080	92	14.77	± .046	34	14.79	± .063	+ .02	± .078
Transverse	♂ 43	1.24	± .007	73	1.77	± .009	41	1.56	± .013	- .21	± .016
Diameter (mm) ..	♀ 42	1.25	± .010	98	1.83	± .012	40	1.53	± .013	- .30	± .018
Sagittal	♂ 42	1.00	± .007	73	1.07	± .006	41	1.13	± .011	+ .06	± .013
Diameter (mm) ..	♀ 43	1.02	± .008	98	1.25	± .012	40	1.15	± .013	- 1.10	± .018
Diametric ratio ..	♂ 42	.799	± .005	73	.606	± .004	41	.725	± .006	- .074	± .008
(Sagittal)											
(Transverse)	♀ 43	.817	± .006	98	.681	± .003	40	.751	± .005	- .066	± .008
Tibia length (mm)	♂ 37	15.31	± .063	67	16.53	± .039	38	16.53	± .050	.0	± .063
	♀ 37	15.17	± .068	94	16.62	± .048	39	16.50	± .050	- .12	± .069
Cranial capacity	♂ 34	4.64	± .034	59	5.31	± .025	38	5.41	± .035	+ .11	± .043
(gms of Hg)	♀ 33	4.70	± .037	70	5.33	± .026	32	5.46	± .037	+ .13	± .045

effects. It might be objected that the influence of heterosis is more marked in skull length and tibia length than in body length and adult weight, or in femur length than in humerus length. Just why, it would be difficult to say. But when heterosis is reduced to a purely genetic basis, it simply means an increase in the number of heterozygous factor pairs in the offspring through different dominant genes brought in by the diverse parents. Wright (1922) and Livesay (1930) have fully discussed the evidence for this view-point. Were the size factors nearly or quite general in their action there seems to be no good reason why an F_1 animal should possess a femur as long as (if a female)—or longer than (if a male)—that of the larger parent while its humerus is significantly shorter. Perhaps a result of disharmonic species crossing!

The nature and relationship of size factors can also be shown to a certain extent by means of correlations between various pairs of quantitative characters. A high coefficient of correlation for a given pair indicates that both are largely subject to common influences or factors. A low coefficient, on the contrary, tends to show that they are largely determined by different factors. Castle (1914, 1922, 1930) has made a number of such correlations for rabbits and found them uniformly high; thus, he believes, demonstrating that size factors in rabbits are, for the most part, general. Wright (1918) in an analysis of Castle's (1914) data by partial correlations has shown that even with the high coefficients of correlation there are factors which influence definite parts of the body independently of general size.

In Table II are presented coefficients of correlation for twelve pairs of characters for each sex of *musculus*, three pairs for *bactrianus*, and the same three pairs for an F_1 generation and for one type of back-cross mating. The types of matings and the two sexes are treated separately since, as Wright pointed out, the inclusion of heterogeneous material would tend to overemphasize the importance of general factors for size.

TABLE II
COEFFICIENTS OF CORRELATION

<i>musculus</i> ♀ × <i>musculus</i> ♂					
Correlations	No.	♂ ♂ Value of <i>r</i>	No.	♀ ♀ Value of <i>r</i>	
1. Skull length—body length	78	+ .601 ± .049	60	+ .745 ± .038	
2. " " —body weight	34	+ .401 ± .097	28	+ .360 ± .111	
3. " " —humerus length ..	76	+ .365 ± .067	86	+ .635 ± .043	
4. " " —femur length	68	+ .323 ± .073	81	+ .602 ± .048	
5. " " —tibia length	75	+ .424 ± .062	90	+ .538 ± .051	
6. " " —skull width	79	+ .386 ± .065	93	+ .397 ± .059	
7. Humerus length—body length ..	83	+ .447 ± .059	59	+ .724 ± .042	
8. " " —body weight	33	+ .220 ± .112	28	+ .615 ± .080	
9. " " —femur length	72	+ .627 ± .049	88	+ .788 ± .027	
10. " " —tibia length ..	81	+ .656 ± .042	98	+ .703 ± .034	
11. Femur length—tibia length	70	+ .761 ± .034	92	+ .818 ± .024	
12. Body length—body weight	35	+ .395 ± .096	32	+ .653 ± .068	
<i>bactrianus</i> ♀ × <i>bactrianus</i> ♂					
13. Skull length—femur length	32	+ .249 ± .112	31	+ .637 ± .072	
14. Skull length—tibia length	35	+ .265 ± .106	33	+ .630 ± .071	
15. Femur length—" "	37	+ .817 ± .037	37	+ .855 ± .030	
<i>musculus</i> ♀ × <i>bactrianus</i> ♂					
16. Skull length—femur length	39	+ .291 ± .099	32	+ .335 ± .106	
17. " " —tibia length	38	+ .415 ± .091	36	+ .407 ± .094	
18. Femur length—" "	38	+ .657 ± .062	32	+ .578 ± .079	
<i>musculus</i> ♀ × <i>musculus</i> ♂					
<i>bactrianus</i> ♂					
19. Skull length—femur length	63	+ .546 ± .060	51	+ .473 ± .073	
20. " " —tibia length	70	+ .723 ± .038	65	+ .284 ± .077	
21. Femur length—" "	63	+ .916 ± .014	52	+ .857 ± .025	

Although the numbers are small in some cases—in consequence of which the probable errors are large—it is nevertheless apparent that there are significant and important differences in the values of the coefficients of correlation between various pairs of characters. Skull length and body length, for example, are more closely correlated than skull length and skull width. Femur length and tibia length are most closely correlated in all types of matings. Castle (1922) found the value of *r* for femur length-humerus length in rabbits to be .91; for tibia-humerus, .90; for femur-tibia, .93. In our *musculus* matings the femur-tibia correlation is consistently—although not in every case significantly—higher than the other two.

TABLE III
COEFFICIENTS OF CORRELATION IN PARENT SPECIES AND HYBRIDS

	1 Femur length— tibia length r	2 Femur length— skull length r	3 Tibia length— skull length r	Difference 1-2 P. E.	Difference 1-3 P. E.	Difference P. E.
<i>musculus</i> ♂	+ .761 ± .034	+ .323 ± .073	+ .424 ± .062	.438 ± .081	.337 ± .071	4.7
♀	+ .818 ± .024	+ .602 ± .048	+ .538 ± .051	.216 ± .054	.280 ± .056	5.0
<i>bactrianus</i> ♂	+ .817 ± .037	+ .249 ± .112	+ .265 ± .106	.568 ± .118	.552 ± .112	4.9
♀	+ .855 ± .030	+ .637 ± .072	+ .630 ± .071	.218 ± .078	.225 ± .077	2.9
F ₁ ♂	+ .657 ± .062	+ .291 ± .099	+ .415 ± .091	.366 ± .177	.242 ± .110	2.2
♀	+ .578 ± .079	+ .335 ± .106	+ .407 ± .094	.243 ± .132	.171 ± .123	1.4
Back-cross ♂	+ .916 ± .014	+ .546 ± .060	+ .723 ± .038	.370 ± .062	.193 ± .040	4.8
♀	+ .857 ± .025	+ .473 ± .073	+ .284 ± .077	.384 ± .077	.573 ± .080	7.2

TABLE IV
SEX DIFFERENCES IN *musculus* ♀ × *musculus* ♂ MATINGS

Correlations	Value of r ♂ ♂	Value of r ♀ ♀	Difference	Difference P. E.
Skull length-humerus length	+ .365 ± .067	+ .635 ± .043	.270 ± .080	3.4
“ “ -femur length	+ .323 ± .073	+ .602 ± .048	.279 ± .087	3.2
Humerus length-body length	+ .447 ± .059	+ .724 ± .042	.277 ± .072	3.8
“ “ -body weight	+ .230 ± .112	+ .615 ± .080	.395 ± .138	2.9
“ “ -femur length	+ .627 ± .049	+ .788 ± .027	.161 ± .056	2.9

When the femur-tibia correlations are compared with femur-skull and with tibia-skull it is evident that the coefficients for the first pair are much higher than the values for the latter two. This is shown in Table III. This seems to demonstrate that the factors determining skull length in mice are not entirely the same as those determining the length of femur or tibia; while the two leg bones do form a group largely subject to the same genetic influences. This is in line with Wright's analysis (cited above) of Castle's rabbit material.

In Table I it was shown that there were significant sex differences in the means of several of the size characters investigated. Female *musculus* are significantly longer bodied than males and have longer skulls and longer femurs with greater transverse and sagittal diameters. The diametric ratios of the females also average higher. There are likewise sex differences in the degree of correlation between various pairs of characters. Of the twelve pairs of correlated characters in *musculus*, eleven show higher coefficients of correlation for females than for males. In Table IV are listed five pairs for which the differences approach significance. This tendency may mean that in female mice general size factors have more influence than they do in males. Merriam (1896) in a taxonomic revision of the weasels of North America stated that among mammals the female is less specialized than the male and consequently bears more resemblance to the ancestral stock. Perhaps in mice specialization also extends to genetic size factors.

SUMMARY

Data on quantitative characters, derived from a mouse species cross, have shown that the F_1 means fall into two general classes: one in which the means are intermediate between those of the two parent species and the other in which they equal or surpass the larger parent. In general, external dimensions (body length, tail length) and weight fall into the first; while skeletal dimensions fall

into the second. Humerus length, however, is intermediate. These results make it appear probable that not all size characters are equally subject to the influence of general factors.

Coefficients of correlation were computed for various pairs of characters and disclosed significant differences. Femur and tibia are more closely correlated than is either with skull length. These two leg bones in mice, as well as in rabbits, are apparently largely influenced by common factors. Sex differences were found in the degree of correlation between various pairs. In general, females present higher coefficients than males perhaps indicating that general size factors play a more important rôle in the former.

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A MUTUAL TRANSLOCATION INVOLVING THE FOURTH AND THE X-CHROMOSOMES OF DROSOPHILA

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INTRODUCTION

NUMEROUS instances of broken chromosomes and translocated fragments have been reported. Cases described previously for *Drosophila* have involved breakages of the X-, Y-, II- and III-chromosomes. This paper presents evidence of the first demonstrated breakage of the IV-chromosome.¹

ORIGIN OF STOCK

The stock, known as mottled 5, was produced by Dr. Muller in 1929 by X-raying a male carrying a normal X-chromosome. It was inferred to involve a breakage and translocation of the X to an autosome on account of flies of characteristic hyperploid appearance produced by it (Muller, 1930). Males possessing the treated X-chromosome exhibit mottled eyes. Females show the mottledness when they have, in addition to the treated chromosome, the gene for white in the other X-chromosome. The genes for vermilion and forked were subsequently placed in the treated chromosomes by crossing-over. The $w^{m5}vf$ strain has been used almost exclusively in these experiments because of the convenient markers, vermilion and forked.

ANALYSIS OF STOCK

(a) *Translocation of X-fragment to IV*

Since the mottledness of the eyes never appears in females heterozygous for the normal allelomorph of white, but does show if a light allelomorph (*e.g.*, white or eosin) is present, it is evident that the locus of white

¹ I express my thanks to Dr. H. J. Muller for suggestions and guidance in attacking this problem.

in the X-chromosome is the one primarily involved in the mottling. Tests show that mottledness is linked to the IV-chromosome. When w^{ms} females were mated to bent males and the F_1 females backcrossed to bent males, both mottled males and bent males appeared in the F_2 but there were no mottled bent males. Subsequent tests proved the loci of yellow and scute were likewise linked to IV but that facet and miniature were not.

Hyperploid males were produced by mating $w^{ms}vf$ males to yw females. From a few such crosses there were obtained a total of 237 gray mottled females, 203 yw males and 45 gray mottled males. The F_1 mottled males undoubtedly received their X-chromosome from the female parent. Had they been non-disjunctional progeny they should have shown vermilion and forked, for these characters could not have been eliminated by crossing-over in the male parent. Hence the mottled males carry an entire yw X-chromosome. But inasmuch as they are gray and mottled they also contain a portion of an X which includes the loci of yellow (gray) and white (mottled). This fragment could have come only from the male parent. Obviously these mottled males are hyperploid.

Some of the hyperploid males show abnormal venation, usually a projection on the posterior crossvein; the hind legs are sometimes deformed; often the wings are spread. The viability is about 20 to 25 per cent. of the yw males. In these respects they agree with other hyperploids carrying as excess the left end of the X. The hyperploid males breed although the fertility is less than normal.

Hyperploid females may be obtained and recognized by backcrossing the hyperploid males described above to yw females. One such cross yielded the following offspring: 97 yw females, 73 yw males, 49 gray mottled females and 20 gray mottled males. The yw flies are non-hyperploid; the gray mottled ones have received the extra fragment. Some of the hyperploid females exhibit venation disturbances.

Hyperploid males obtained by mating $w^{m5}vf$ males to yellow white facet females are gray mottled facet; those from yellow scute white miniature females are gray non-scute mottled miniature. The conclusion is that the X-chromosome is broken between the loci of white and facet and that the left end is attached to the IV-chromosome. The translocation has been termed "X-IV2," as it is the second case of breakage of the X and its attachment to chromosome IV. For the first case see Muller and Stone (1930) and for the third case Patterson and Painter (1931).

(b) *Translocation of IV-fragment to X*

The $w^{m5}vf$ males were mated to yellow attached-X eyeless females. The F_1 $w^{m5}vf$ males were backcrossed to yellow attached-X eyeless females. The F_2 females were yellow eyeless, gray eyeless, yellow non-eyeless and gray non-eyeless. The F_1 males are all non-eyeless. The gray females carry the IV to which the left end of the X is attached as shown by the fact that they are non-yellow. Two alternative explanations of the presence of eyeless in part of the gray females arise. The first is that a mutation to eyeless has occurred in the IV to which the X-fragment is attached. This is obviated by the fact that the $w^{m5}vf$ males are all non-eyeless.

The second alternative assumes that the IV-chromosome is broken and that a portion carrying the normal allelomorph of eyeless is attached to the right end piece of the broken X. This will account for the eyeless condition of some of the F_2 gray females mentioned above. They had received an eyeless IV from the mother and the IV carrying the X-fragment from the father. If the locus of eyeless is absent from this paternal IV, then the appearance of eyeless in the F_2 gray females is to be expected. The F_2 $w^{m5}vf$ males are all non-eyeless because they possess, in addition to the part of the IV to which the X-fragment is attached, that portion of the IV containing the normal allelomorph of eyeless. This frag-

ment of the IV can not be attached to the Y-chromosome, for the Y-chromosome came from a mother of normal IV ancestry. If it were attached to either of the large autosomes only half of the F_2 , $w^{m5}vf$ males would have received it and have been non-eyeless. Therefore the IV-fragment must be attached to the right portion of the X-chromosome but to the left of vermilion, for vermilion and forked got into the chromosome by crossing-over after the x-ray treatment.

(c) *Non-disjunction of IV*

In the F_1 of the cross discussed above all the flies were non-eyeless although the gray females might be expected (on the present hypothesis) to show eyeless. When these F_1 gray females were mated to $y\ sc\ w\ ey$ males the progeny were as follows: yellow, yellow eyeless, gray, gray eyeless females and yellow scute white, yellow scute white eyeless, gray mottled, gray mottled eyeless males. It is evident that some of the gametes carrying the gray mottled containing fragment must also have carried the normal allelomorph of eyeless. This has occurred through non-disjunction of the IV. The fragmented and attached condition of the IV probably would hinder perfect synapsis and result in a high degree of non-disjunction. Apparently an eyeless fly which receives only one entire IV and the fragment of the IV not containing the locus of eyeless has low viability.

Various crosses were designed and carried out which should have given eyeless flies in the F_1 if IV-hypoploid eyeless flies are viable, but no eyeless offspring appeared. Special effort was made to furnish optimal cultural conditions for the test. Yellow attached-X eyeless females were mated to $w^{m5}vf$ males. From three cultures there were obtained 81 $w^{m5}vf$ males, 74 yellow females and 46 gray females. None of the gray females was eyeless although all had received an eyeless IV from the mother and from the father the portion of the IV which lacks the locus of eyeless. It seems evident that through non-dis-

junction a normal IV had accompanied the IV-fragment from the male parent. When $w^{ms}vf$ males were crossed to $y\ sc\ w\ ey$ females the following progeny were secured from ten cultures: 381 gray mottled females, 253 $y\ sc\ w$ males and 57 gray mottled males. Again, the failure of gray mottled eyeless males to appear indicates a very low viability (perhaps inviability) of eyeless flies haploid for the portion of the IV containing the locus of eyeless.

There is cytological evidence of non-disjunction of IV in mottled 5. Dr. Bessie League (unpublished data) found some cells containing what were apparently three IV-chromosomes, and other cells with four. The translocated X-fragment has not been detected, presumably due to its small size. In this connection compare the case reported by Patterson and Painter (1931).

DISCUSSION

Flies hypoploid with respect to the X-chromosome have not been discovered in the w^{ms} stock. When $w^{ms}vf$ males are mated to yw females all the female offspring are gray mottled. Females lacking the left end fragment of the X do not live even though they possess one complete X-chromosome. This harmonizes with Patterson's demonstration of a viability gene in the left end of the X-chromosome (Patterson, in press).

The evidence indicates that the IV-chromosome is broken between the locus of eyeless and the point of attachment of the spindle fiber. I propose to refer to the locus of eyeless as being to the left of the spindle fiber. It seems clear that in the left end fragment there is a gene or genes, the double dose of which is important for the viability of the fly, at least when the latter has the gene for eyeless and not for its normal allelomorph.

Tests with bent similar to those performed with eyeless indicate that it is in the right end portion of the IV. Other fourth chromosome genes may be tested to determine their locations with respect to the break and thus

a partial map of the IV-chromosome may be constructed. As shown by work of Morgan, Sturtevant and Bridges (1926), and of Patterson and Muller (1930, see pp. 523-524) previously published "maps" of this chromosome, based upon supposed crossing over (Morgan, *et al.* 1925) are invalid.

SUMMARY

(1) The $w^{ms}vf$ stock of *Drosophila melanogaster* contains a mutual translocation of X and IV, produced by x-ray.

(2) The X-chromosome is broken between white and facet; the left end is attached to the IV.

(3) The IV-chromosome is broken between eyeless and bent and the eyeless (left) part is attached to the right portion of the broken X. Bent is located on the spindle fiber (right end) portion of the IV.

(4) Non-disjunction of IV occurs very frequently in this stock.

(5) A gene, the double dose of which is important for viability, is located in the left (eyeless) end of the IV.

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THE WEIGHT-LENGTH RELATIONSHIP OF THE SHELLS OF THE HAWAIIAN PEARL OYSTER, *PINCTADA SP.*¹

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SINCE the formulation of Spencer's "cube law" (1872) stating that the weights of the organisms increase as the cubes of their dimensions, numerous attempts were made to apply this relation to various animals and to fit the observed and calculated data to a simple formula, $W = aL^3$, where W is weight, L is length and a is a constant dependent on the unit of measure and the organism studied. This simple formula was often used in a study of growth and in comparing the weight-length factors, $a = \frac{W}{L^3}$, of various food fishes. An extensive and widely scattered bibliography on the subject compiled by Clark (1928) can be found in her paper on the weight-length relationship of the California sardine. Although the application of a "cube law" gave satisfactory results for fishery studies (Hensen, 1899; Heincke, 1908; Johnstone, 1914), its validity and theoretical significance for the interpretation of the relation between growth and form has been but little studied. In 1916 Hecht, accepting the cube law as representing the true relation between the weight and the length, came to the conclusion that in the organisms in which growth is uniform but unlimited (indeterminate) the form of the individual is laid down very early in life and is adhered to throughout its period of growth which lasts for the rest of its life (fishes, bivalve mollusc *Dosinia*), whereas in the organisms with determinate growth (man) the form is continually changing during the period of growth. In the latter case when the form becomes constant the growth ceases. In recent years Huxley (1924, 1927) has developed this idea more

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elaborately and arrived at the general formula which describes both the "isogonic" growth, when the part bears a constant relation to the whole, and the "heterogonic" growth when a part of the body is increasing or decreasing in relation to the whole.

The works of Clark (1928), Duncker (1923), Keys (1928) have shown that the cube law fails to express the exact weight-length relationship and that the latter could be described by a more general equation $W = aL^n$ where the value of n , computed from the measurements, is ordinarily greater than 3 and less than 4. The applicability of this formula to the weight-length relationship in molluscs *Sphaerium*, *Littorina*, *Limnaea* and *Viviparus* has been demonstrated by Nomura (1926).

In July-August, 1930, the author collected 164 live specimens of adult pearl oyster, *Pinctada sp.*² at Pearl and Hermes reef, a small atoll of the Hawaiian archipelago located between the latitudes 27°45' and 27°58'N and longitudes 175°43' and 176°00'W. Besides adult oysters, varying in size from 10 to 29 centimeters, the collection comprised 33 small oysters from 0.3 to 3.5 centimeters long which were found attached to the shells of the adult ones. They undoubtedly represented the newly hatched oysters that have just attached themselves. Oysters of intermediate size, from 6 to 10 centimeters long, were absent. This suggests that the spawning of the pearl oyster in Pearl and Hermes reef occurs only during the summer. Physiological and ecological observations which will be published elsewhere corroborate this conclusion.

Inasmuch as the rate of growth of the oyster is not known, there is no means of determining the age of the

² In the National Museum in Washington and in the Bishop Museum at Honolulu the Hawaiian pearl oyster is listed under the name *Pinctada cumingi* Reeve. A careful examination of the material collected in the Hawaiian Islands has convinced me that the Hawaiian species differs from the characteristics given by Reeve and referring to the oyster from the Paumotu Islands. A detailed description of the Hawaiian species will be given elsewhere.

individuals of various sizes. It is very likely, however, that the one year old oyster is about 13 centimeters long. This conclusion is based on the measurement made on one specimen which was planted in the summer of 1929 by one of the divers employed by the author, and was found again in August, 1930. According to the testimony of the diver the oyster, when planted, was about one inch long. During July and August, 1930, all the oysters of 10 centimeter size or larger contained fully developed gonads and were ready to spawn.

In the following discussion the length, L , is regarded as the greatest dimension of the shell measured at a right angle to the hinge side. The width, W , is the greatest distance across the shell at a right angle to the length. Both length and width were measured to the nearest half centimeter. The weight of the shells, dried at room temperature, was determined to the nearest gram. Figures of weight, given in the text, indicate the weight of both valves of the oyster. In measuring and weighing

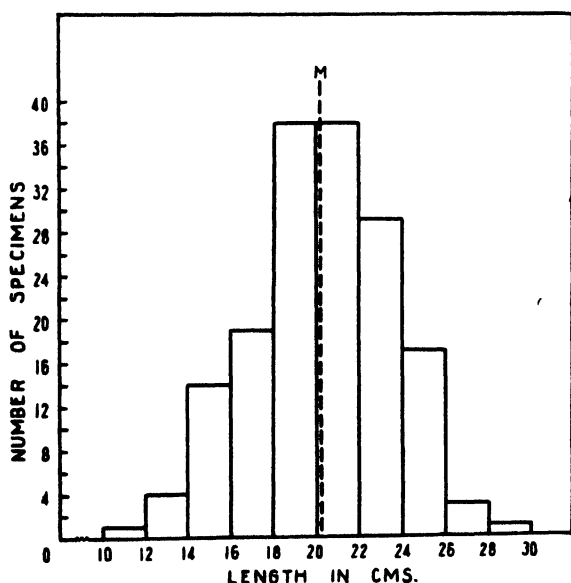


FIG. 1. Frequency distribution of the length of the adult pearl oysters. (Small oysters less than 6 cms long are not included.)

small oysters the second decimals of a centimeter or of a gram were read.

The frequency distributions of the length and weight of the adult oysters (Figures 1 and 2) indicate that specimens between 18 and 22 cms long and weighing from 400 to 600 grams were predominant. It is interesting to note that the frequency distribution of the length (Fig. 1) gives a more symmetrical curve than that of the

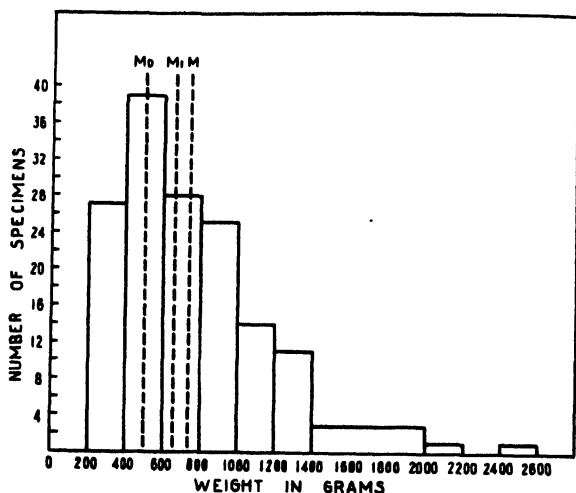


FIG. 2. Frequency distribution of the weight of the adult pearl oysters. (Small oysters less than 6 cms long are not included.)

weight (Fig. 2). The probable reason of such a difference will be discussed later.

The biometrical constants for the length and weight of the shells of the adult oysters are given in Table I.

TABLE I

BIOMETRICAL CONSTANTS FOR THE LENGTH AND WEIGHT OF THE SHELLS OF THE ADULT *Pinotada* sp.

	Mean	Pr. err.	St. deviation	Median	Mode
Length cms	20.2	± 0.17	3.24 ± 0.12	20.3 ± 0.21	20.5
Weight gms	734	± 22.6	418 ± 15.6	650 ± 28.2	492.6

A frequency distribution of the weight of the adult oysters, grouped in six classes according to their length

(Fig. 3) shows that the skewness of the variation curves increases with the increase of length (compare the classes marked 21, 23 and 25 with those of 15, 17 and

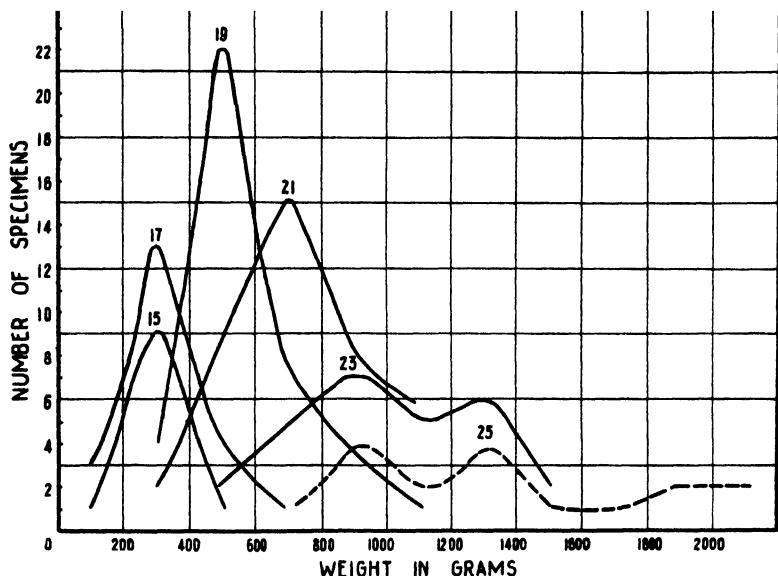


FIG. 3. Frequency distribution of the weight of shells in six classes of pearl oysters of various lengths. The figures in the body of the graph indicate the length of shells in cms. Because of a small number of observations the shape of the curve of class 25 (dotted line) can not be regarded as significant.

19). This fact, coupled with the observation that the frequency curve of the weight of the oysters (Fig. 2) is less symmetrical than the frequency curve of their length (Fig. 1), can be interpreted as indicating that the deposition of shell substance, or in other words, the increase in the thickness of the shell, continues in spite of the retardation or cessation of the growth of shell in length.

The biometrical constants for the weight of the oysters of various classes are given in Table II.

In computing the mean for the class of oysters 14–15.9 cms long one measure of 697 grams was rejected because its deviation from the mean was 10.3 times the standard error. The probable error of the mean of the class of

TABLE II
BIOMETRICAL CONSTANTS FOR THE WEIGHT OF VARIOUS CLASSES OF
Pinotada sp.

Length cms.	Number o specimens	Mean grams	Probable error	St. de- viation	Median	Mode	Coef. of variation per cent.
0- 1.9	22	0.21	± 0.02	0.15 ± 0.02	0.20 ± 0.03	0.20	66.1
2.0- 3.9	10	1.33	± 0.14	0.61 ± 0.09	1.17 ± 0.17	0.85	45.9
4.0- 5.9	1	(3.5)					
10.0-11.9	1	(68)					
12.0-13.9	4	163	± 17.5	37.5 ± 8.9			23.0
14.0-15.9	14	262	± 15.9	81.4 ± 10.8	258 ± 19.9	250	31.1
16.0-17.9	19	375	± 17.3	109 ± 11.9	356 ± 21.7	318	29.1
18.0-19.9	38	570	± 22.3	201.5 ± 15.6	522 ± 27.6	426	35.4
20.0-21.9	38	738	± 23.1	208 ± 16.1	729 ± 28.5	711	28.9
22.0-23.9	29	998	± 36.8	289 ± 15.6	925 ± 46.2	779	29.0
24.0-25.9	17	1265	± 60.8	361 ± 41.8	1275 ± 76.1	1295	28.8
26.0-27.9	3	(1849)					
28.0-29.9	1	(2509)					

Mean computed from a small number of observations is shown in parentheses.

12-13.9 cms was corrected for the small number of observations ("Student," 1925). An examination of the columns of standard deviations and of the Pearsonian coefficient of variation reveals the extent of variation in weight within each class. The coefficient of variation of small oysters (first two classes) is much higher than in the adult ones, but the differences in the coefficients of variation of larger oysters can not be regarded as significant. The highest variability probably corresponds to the period of most rapid growth (Weymouth, 1931) which, judging from our knowledge of the growth of the edible oyster (*Ostrea*), occurs immediately after the attachment of the larvae.

In spite of a great variability of weight within each class the mean weight of the oysters increases at a definite rate with the increase in length. Excepting the first class (oysters less than 2 cms long) and the two largest

classes (from 26 to 29.9 cms long), which are represented by a very small number of observations, the relation between the logarithm of weight and the logarithm of length can be represented by a straight line (Fig. 4).

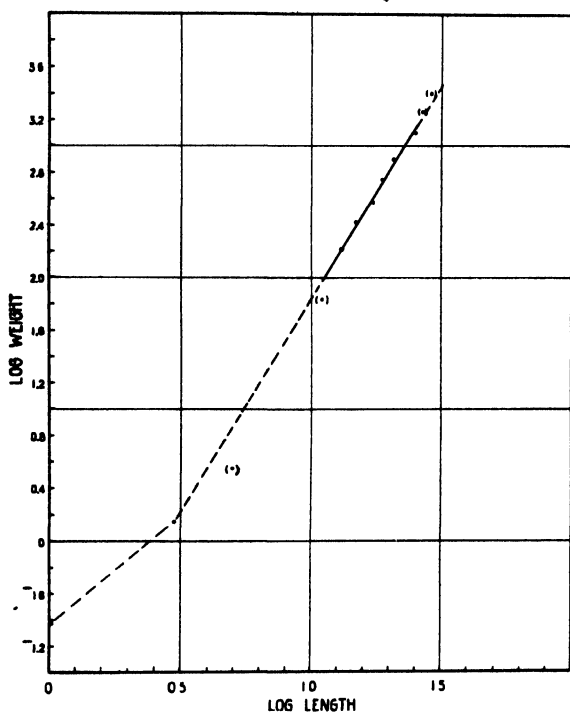


FIG. 4. The relationship between the log. of length and the log. of weight of shells.

Mathematical analysis of the curve reveals that within the limits just mentioned the weight-length relationship can be described by the formula $W = aL^n$. Determining the values "a" and "n" by the method of least squares we arrive at the following equation:

$$W = 0.04209 L^{3.21529} \quad (1)$$

The curve representing this equation is shown in Fig. 5. As one can notice from an examination of Fig. 5 and Table 3, whenever there is a sufficient number of observations, the observed mean values (triangles) fit the computed figures (circles) very well.

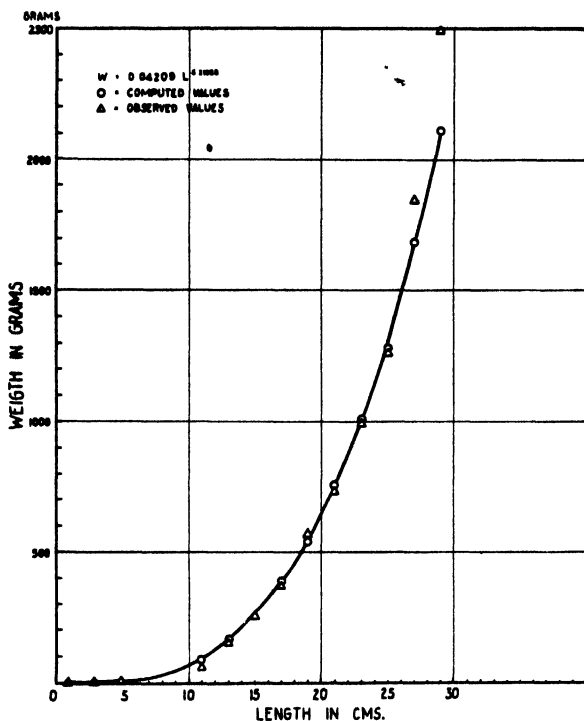


FIG. 5. The relationship between the length and the weight of shells.

TABLE III

OBSERVED AND CALCULATED VALUES OF THE WEIGHTS OF THE SHELLS OF
Pinctada sp.

Length in cms.	Number of specimens	Observed mean weight grams	Calculated weight grams	Difference between calculated and observed weight
0.0- 1.9	22	0.21	0.04	- 0.17
2.0- 3.9	10	1.33	1.44	+ 0.11
4.0- 5.9	1	3.5	7.4	+ 3.9
10.0-11.9	1	68.0	93.9	+ 25.9
12.0-13.9	4	163.0	165.6	+ 2.6
14.0-15.9	14	262.0	254.5	+ 2.5
16.0-17.9	19	375.0	380.5	+ 5.5
18.0-19.9	38	570.0	544.1	- 25.9
20.0-21.9	38	738.0	750.7	+ 12.7
22.0-23.9	29	998.0	1,005.7	+ 7.7
24.0-25.9	17	1,265.0	1,331.7	+ 66.7
26.0-27.9	3	1,849.0	1,684.5	- 165.5
28.0-29.9	1	2,509.0	2,110.9	- 388.1

From the fact that during the period of growth from 2 to 25.9 cms the mean weight of the shell bears a constant relation to its length the following inferences can be made: that within this range the growth is not accompanied by a change in form, or if there is any change in form it is counterbalanced by the change in the specific gravity of the shell material. The second assumption is very improbable and is not confirmed by the examination of the shells. Inasmuch as the shell of the pearl oyster is flat and round the changes in its form could be easily expressed by the length-width ratios or by the percental width ($100 \frac{W}{L}$). Table 4 comprising these data shows that no significant changes occur in the shape of the

TABLE IV

MEAN VALUES OF THE LENGTH-WIDTH RATIOS AND PERCENTAL WIDTH OF THE SHELLS OF *Pinctada* sp.

Length cms	Number of specimens	Mean ratio $\frac{L}{W}$	100 $\frac{W}{L}$
0.0- 1.9	22	1.015±0.02	98.5
2.0- 3.9	10	1.02 ± 0.03	98.0
4.0- 5.9	1	(1.05)	(95.2)
10.0-11.9	1	(1.05)	(95.2)
12.0-13.9	4	1.16 ± 0.02	86.2
14.0-15.9	14	1.17 ± 0.02	85.5
16.0-17.9	19	1.10 ± 0.01	91.2
18.0-19.9	38	1.17 ± 0.02	85.5
20.0-21.9	38	1.13 ± 0.02	88.5
22.0-23.9	29	1.19 ± 0.02	84.0
24.0-25.9	17	1.22 ± 0.03	82.0
26.0-27.9	3	(1.15)	(87.0)
28.0-29.9	1	(1.28)	(78.1)

adult oysters (from 10 to 23.9 cms long). It has been found that oysters of the class 24-25.9 cms were distinctly longer (ratio 1.22), but unfortunately an insufficient number of measures in the next larger classes makes it impossible to decide whether the difference is

significant. Small immature oysters are decidedly shorter than the adults (ratios 1.015-1.02). This confirms the conclusion reached from the examination of the weight-length curve that during the period of growth the young oysters change in shape. After they have reached 10 cms in length their form remains nearly constant.

From the analysis of the weight-length relation of shells of the Hawaiian pearl oyster a conclusion can be reached that there is no sharply defined difference between the determinate and undeterminate growth and that the growth of immature oysters is accompanied by a gradual change in their form.

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THE YAQUINA OYSTER BEDS OF OREGON

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IN connection with an investigation of the oyster beds of the Yaquina Bay and River regions of Oregon certain general biological features were revealed which are of interest to students of oyster culture, particularly those who have considered the oyster producing areas of the Pacific Coast. The investigations referred to were conducted chiefly during the fall and spring months of 1927-1928, and embraced that portion of the Yaquina River between the towns of Newport and Elk City, a stretch of over twenty miles. Since then, visits have been made at irregular intervals to this vicinity, and they have revealed that with minor differences conditions at the present time are much the same as when the intensive investigations were conducted some three years ago.

THE YAQUINA RIVER

The Yaquina River is a small, narrow stream lying about 100 miles south of the Oregon-Washington boundary, running for a distance of about thirty or more miles inland, beginning at its mouth on the Pacific Ocean and passing along its course the settlements of Newport, Yaquina, Oysterville, Toledo and Elk City. Newport is situated at the mouth of the river and Toledo is approximately eleven miles distant from it. Between these two places and for another mile above the city of Toledo, the stream is really an estuary (Fig. 1).

As mentioned, the river is narrow, and it varies in width from about 250 yards at Toledo to nearly 700 yards below Yaquina, where it enters Yaquina Bay, upon which the main part of the city of Newport is located. Above Toledo, the stream becomes much narrower, so much so that around Elk City it is no more than 100 feet across. Moreover, its main channel is quite shallow, varying in

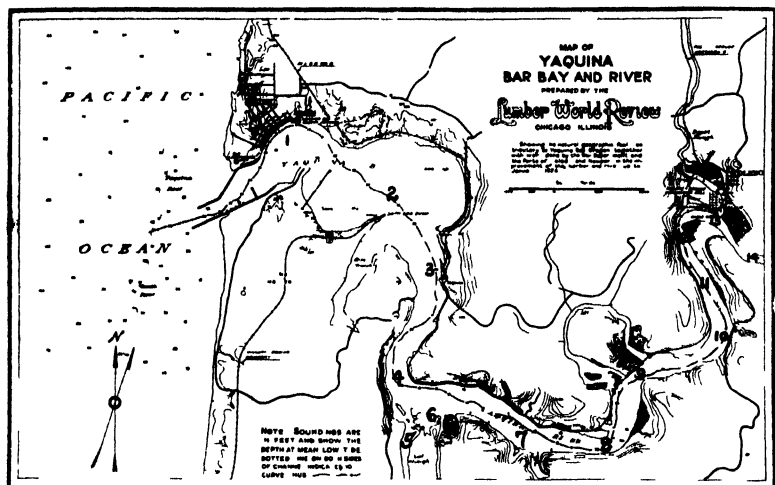


FIG. 1. Map of Yaquina River region.

depth from approximately ten to thirty feet. The river flow is controlled by the ocean tides, the average difference between the rise and fall of the waters being around eight feet. This tends to scour and cleanse the stream daily. The banks contain large quantities of sand and mud, and owing to the tides these materials are being shifted around constantly. The mouth entrance to the Yaquina River is forever changing, so much so that on all maps issued by the United States Coast and Geodetic Survey the following caution is invariably given: "The entrance to Yaquina Bay is changing. Strangers should not attempt to enter without a pilot."

The shores along the river bank are quite steep, in many places rising to a height of over 500 feet. They are covered with vegetation, sand, gravel and stone. Rains and storms, which are especially prevalent here during the fall, winter and early spring months, wash large quantities of leaves, twigs, trees, shrubs and land deposits into the waters, thereby adding to the quantities of solid particles brought down from the upper parts of the stream with the ebb tide and washed up from the ocean below with the flood tide. The stream is thereby filled with tremendous quantities of floating silt, mud and

débris, so that the ebb and flow of the tides create currents which carry these suspended particles up and down the river bed, changing and shifting it accordingly. This has been going on for years, and it has made it difficult to maintain channels for navigation. Furthermore, it has interfered with the natural oyster beds located at the small settlement of Oysterville, approximately six miles below Toledo, mainly on the south shores of the river, and extending for a distance of about three miles in a direction towards the mouth of the stream. This is shown on map, Fig. 1, between stations 3 and 8. Fig. 2 shows their exact location.

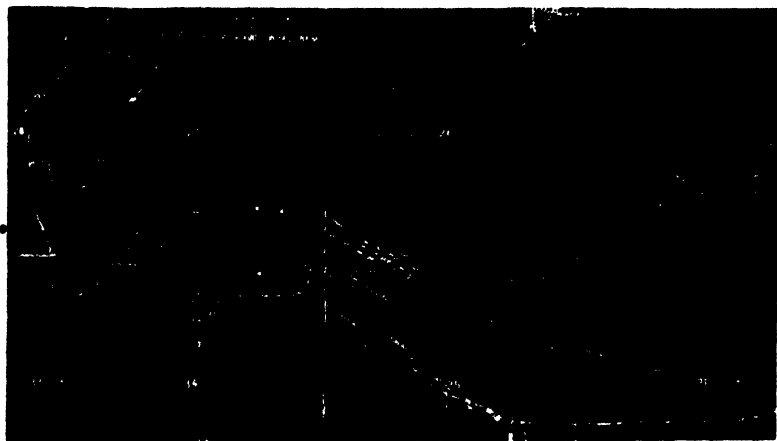


FIG. 2. Map of Yaquina oyster beds as surveyed December 1, 1908.

That this has been the case is a matter of official record, for in the year 1917, Charles H. Edmondson, special investigator for the U. S. Bureau of Fisheries, visited the region and has written a bulletin entitled "Shellfish Resources of the Northwest Coast of the United States," Appendix III to the Report of the U. S. Commissioner of Fisheries for 1922, in which he tells us the following regarding the oyster beds of the Yaquina: "Since the beds are, for the most part, located in the channel of the river, in 1917 the fishermen on the artificial plantations were having considerable difficulty, as

had been the case in previous years, in preventing the oysters from being smothered by the sediment carried down the river. Frequent tonging was resorted to to keep the ~~till~~ moving. Little or no artificial methods were resorted to in order to catch the spat. The old shells were considered, by those engaged in the industry, as sufficient catch, with the occasional help of the bark of trees."

* During one of my visits to this region, on November 16 and 17, 1927, the Yaquina River between Newport and Toledo was so muddy that it was completely turbid, brownish and densely opaque in appearance. The reason for this state of affairs was the terrific storm which visited the vicinity the previous day, Tuesday, November 15, when a fierce gale, blowing about seventy miles an hour, coupled with torrential rains, washed tremendous quantities of material from the shores into the stream. As already mentioned, during the late fall, winter and spring months quite a number of similar storms occur in this region so that the Yaquina River often takes on such an appearance. Large quantities of mud, silt, ooze, leaves, branches, bark, pebbles and other débris are thereby scattered far and wide, back and forth over the stream.

Into this stream, also, human sewage enters at various points. The city of Toledo empties practically all of its sewage, and otherwise, directly into Depot Slough and from here it ultimately finds its way into the Yaquina River. The main business section of Newport dumps all of its sewage and other waste into Yaquina Bay, from where it is swept back and forth over the course of the river. Between Newport and Toledo there are smaller settlements such as Yaquina and Oysterville, and isolated boat houses as well as shore homes, which also pollute the stream with human sewage. Above Toledo a similar condition prevails, with the result that large quantities of untreated human sewage and other wastes of one kind and another, particularly from dairies, garages and boat-oiling stations, find their way di-

rectly into the stream. The tides then carry such material back and forth over the entire river, making it possible for dangerous disease-producing organisms to be disseminated to those forms which dwell in the stream and are utilized by man for food purposes. Inasmuch as the oysters from the Yaquina region are sent to various markets, mainly those of the Portland district, for human consumption, they may be regarded as a source of grave danger to human individuals.

GENERAL EXTENT OF OYSTER BEDS

The oyster beds on the Yaquina River are located around the settlement of Oysterville, situated mainly on the south banks of the stream, beginning a little above the town of Yaquina and extending for a distance of about three miles towards Toledo. Fig. 2 is a photograph of an official map of the oyster beds as they existed in 1908, the last time that they were surveyed and recorded. Fig. 3 shows the shore of Oysterville as it ap-



FIG. 3. Shore at Oysterville.

pears at the present time. Eastern oysters have been planted here during the last few years.

Harlan B. Holmes, of the U. S. Bureau of Fisheries, who investigated these oyster beds in January, 1927, and

has written an unpublished report on them, describes their location as follows: "Along the lower four or five miles of the river, from near the town of Yaquina to Boon's Point, there are approximately 135 acres of oyster beds. The oysters are of a native variety which is somewhat larger than the well known Olympia oyster of Puget Sound. About one-half of the beds are the property of the State of Oregon. The remainder formerly were owned by a number of individuals, but several years ago a company, the Oregon Oyster Company acquired title to the privately owned beds and leased the public beds from the State." Holmes' description gives in comprehensive manner the extent of the oyster beds on the Yaquina River.

During the investigation numerous visits were made to the area and large quantities of oysters were obtained from: (1) the beds of Mr. George Lewis (station 5, Fig. 1), the only privately operated beds which are under the control of the Oregon Oyster Company, (2) the planted beds of the Oregon Oyster Company between stations 6 and 7, Fig. 1 and Fig. 3), and (3) the most exclusively native Atlantic oysters which are shipped in from the Atlantic coast, and (3) the beds (station 4, Fig. 1), leased by the Oregon Oyster Company from the State Fish Commission. Before these oysters can be discussed it is necessary to have a general understanding of the life and conditions of existence of oysters, particularly the western species.

LIFE-HISTORY OF OYSTER

The oyster which is native to the western coast of North America is the small variety, *Ostrea lurida* Carpenter (Fig. 4), and this is the form which is also found to be native on the beds of the Yaquina River. The eastern oyster found on the Atlantic coast is much larger in dimensions and goes under the scientific name of *Ostrea virginica* Gmelin (Fig. 5). This species is not found to

occur in any of our Pacific waters unless it has been introduced through the agency of man.

Our western oyster, the one which is native in the Yaquina River, has taken on distinctive characteristics which mark it off from its eastern relative. While in general many similar factors of food and environment control the life of both of them, yet there are particular differences which mark them off quite distinctly. To a

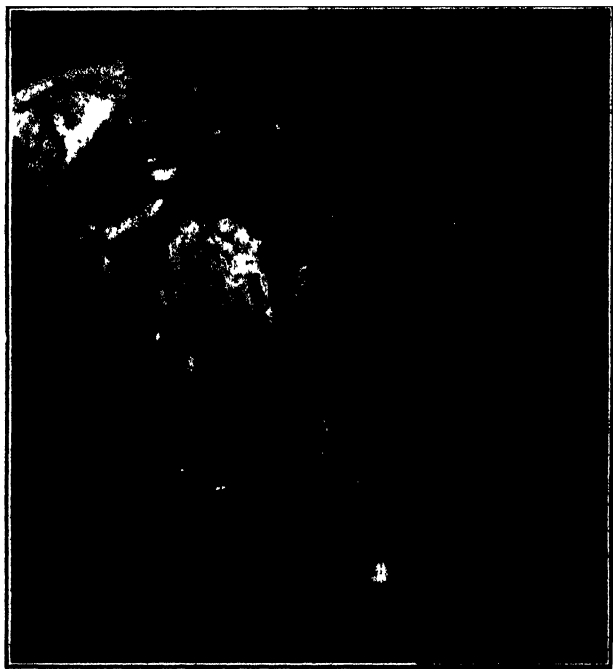


FIG. 4. Native Yaquina oyster, *Ostrea lurida* Carpenter. Note the young spat near lower margin. $\times \frac{1}{2}$.

very large extent we owe our knowledge of the life-history of our native western oyster to the careful studies of Stafford (1913-1917). Our west coast form is hermaphroditic or bisexual, developing both male and female reproductive organs on the same individual. The east coast form is unisexual, being either male or female and not both. Our western oyster, unlike the eastern one, does not shed its germ cells directly into the water,

where fertilization takes place, but the eggs are fertilized within the oyster itself and here they undergo development for some time before they are discharged into the water. Furthermore, the western oysters reproduce



FIG. 5. Transplanted eastern oyster, *Ostrea virginica* Gmelin. $\times \frac{1}{2}$.

young which grow to maturity in our waters. The eastern forms, while they may fatten and grow to enormous sizes and perhaps produce germ cells, do not, as a rule, reproduce young which come to maturity; they are invariably killed off before they proceed very far in devel-

opment. Therefore, from the standpoint of maintaining a constant supply in our western regions, we should be primarily interested in our western oyster, *Ostrea lurida*.

It has been estimated by competent investigators that many of our western oysters attain maturity in one year's time. This period may be compared with the time required for marketing, which has been established definitely that it takes about two years for our western oyster to attain marketable proportions. The breeding season comes on in the spring as early as the latter part of May, but does not reach its height until about the middle of June. Oysters are bisexual, nevertheless the male reproductive organs attain maturity before those of the female. Individual variations take place, of course, so that some oysters mature earlier than others, so that while in some the male organs are ripe, in others the same organs have not attained maturity and the female reproductive organs have not attained ripeness. The mature male oysters discharge into the water and are drawn into the oysters so that in cases where the female reproductive organs are ripe, they become fertilized and start the process into what are known as larvae. The reproductive process thus far described is called "spawning." According to Stafford (1914) it usually lasts from the latter part of May to the last of July, attaining its height in the middle of June.

After fertilization the resultant larvae remain inside the oyster for about sixteen days, and then they are discharged into the water, where they swim freely in a swarm for about fourteen days longer. Soon they settle down on some solid object, permanently, as very young oysters ready for development to adult form. The swimming larvae are spoken of as "free-swimming larvae," and when they settle down on solid objects they are called "spat." The objects to which they attach themselves are known as "cultch," and this material consists of

stone, old shells, bits of wood, bark—in fact, any object which affords a solid surface for attachment. The spat, during the first few months of development, are generally alluded to as “seed oysters,” and these are the ones which are of great importance in repopulating old, depleted beds or starting new ones. In reality spat may vary in size, from seed to the adult form.

After the seed has once started its progress it must then have suitable care and environment for proper development. If the region is one in which there is not enough food material, the young oysters will starve to death. Also, if they become stationed in a locality where there is much sediment, they will be smothered to death. If, on the other hand, the seed oysters are exposed to drying, or to a lack of salts in the water, they will soon perish. Therefore, it is essential that the oyster culturist familiarize himself with the many points connected with the life and environment of his oysters.

FOOD OF OYSTER

In order that oysters may grow normally they must not only find themselves on a suitable bottom, but in a locality which affords them an abundant food supply. The food of oysters consists almost exclusively of minute diatoms, bits of algae, bacteria, Protozoa, and other minute organisms such as crustacean larvae found floating about in the waters over the oyster beds. The main bulk of the food, however, consists of diatoms and plant particles (Fig. 6 and 7). Where stream conditions are favorable these are found in sufficient quantities to maintain the life of the animals. In reality, an examination into the environment in which the oyster lives is a pretty good index as to whether conditions within the stream are suitable for maintaining oysters and other similar animals.

As will be shown later on when the life of the Yaquina is described, the stream contains an abundance of microscopic plants and animals (Fig. 6) floating about within

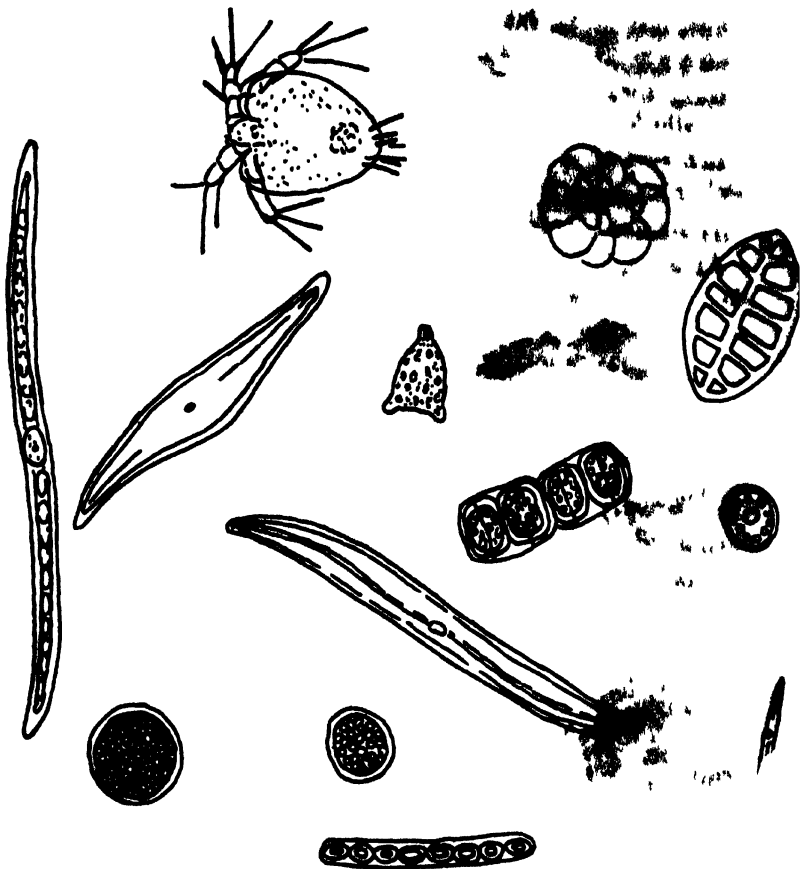


FIG. 6. Plankton organisms in the Yaquina River. $\times 170$.

the water, affording ample food for the oysters. Furthermore, examinations of the contents of the stomachs of many oysters revealed that they are actively feeding on natural food—diatoms, bits of algae and the like—those suspended over the oyster beds. Fig. 7 shows camera lucida drawings of the typical food in the stomachs of Yaquina oysters as revealed by the microscope.

This food is brought into the oyster with water currents that enter the valves. Sand, mud and other particles are also found mixed with the water. The food and bits of organic materials are strained out and then passed to the mouth, where they are taken into the stom-

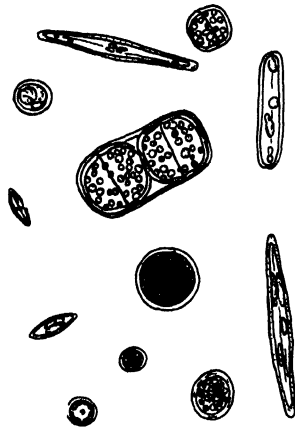


FIG. 7. Food organisms from stomach of native Yaquina oyster. $\times 170$.

ach. The other solid, inorganic particles are left in the general cavity of the oyster enclosed by the gills, and when the valves open they are quickly removed to the outside by the water currents. Examinations of these solid particles showed that they consisted almost exclusively of sand and mud, similar to the bottom upon which the oyster lives.

SALINITY OF WATER

The salinity of the water was determined by the use of a hydrometer or salinometer which indicated its specific gravity. Ordinarily, the specific gravity of pure water is taken as 1. If there is any salt dissolved, the liquid will be heavier than 1 and the difference between the two fluids shows the amount of salt present. Students of oyster culture have found that waters which vary in specific gravity between 1.008 and 1.024 afford the most desirable localities for oyster beds. In actual practice the best regions have been found to be located between the ranges of 1.012 and 1.020.

In an endeavor to determine the salinity conditions of the waters of the Yaquina River, a number of hydrometer readings were made at various points along the stream on several occasions. The results in each case were approximately similar to those obtained on Satur-

day, October 15, 1927, which are listed below. On this date the determinations were made at ebb tide. By referring to the map, Fig. 1, the stations as given in Table I can be located, at which places specific gravity readings were made.

TABLE I

Station	Place	Specific Gravity
13	Near Toledo	1.004
8	Above Oysterville (Termination of oyster beds)	1.012
7	At Oysterville	1.014
6	In front of Pool's Slough	1.015
5	In Pool's Slough	1.012
4	Below Pool's Slough (Beginning of oyster beds)	1.019
3	At Yaquina	1.022
2	Yaquina Bay	1.024
1	Newport	1.028

The oyster beds, as already mentioned, are situated between stations 4 and 8, and the specific gravity or salinity of the water here varies from 1.012 to 1.019, showing definitely that in this location the water has appropriate amounts of salt for the best development of oysters.

LIFE IN YAQUINA RIVER

Just as the barometer indicates the climatic conditions of the environment, so also does the life in a stream indicate whether conditions are wholesome within it. In attempting to ascertain the conditions which surround the oyster beds, particular attention was paid to the life which exists at the present time within the river. The assumption which guided this investigation was that if the stream possessed a normal and healthy life, then the probability is that conditions are normal. On the other hand, if life is scarce, then one would be justified in concluding that conditions are unfavorable within the stream.

That portion of the stream between Toledo and Oysterville was most carefully investigated, and here the life was found to be abundant, vigorous and perfectly normal. The surface catches of plankton as revealed through microscopic studies of the contents showed large numbers of diatoms, algae, Protozoa, minute Crustacea, particularly copepods, and numerous larvae of such crustacean forms as crabs, barnacles, copepods and the like.

The bottom and shore showed a large variety of different organisms. There were numerous Crustacea such as free-living crabs, hermit crabs, barnacles and beach fleas (amphipods and isopods). Buried in the mud were found numerous sand and tube worms. Molluscs also abounded here, there being sea-snails (*Thais*), sessile barnacles, mussels, clams, cockles, oysters, and jelly-like egg masses of snails deposited on various sea weeds. Fig. 8 shows some of the common species of molluscs. Many of the molluscs had sea weeds and bryozoan sea mats covering their surfaces. Here also were found numerous sea anemones, hydroids and sponges. When a microscopic examination was made of the muddy bottom of the river, an abundance and variety of minute forms, such as Protozoa, diatoms and fragments of algae could be distinguished.

At the time when the investigation was being conducted large numbers of salmon of various kinds were running up the river, and fishermen all agreed that it had been one of the most prosperous seasons for salmon fishing that they had known in years. Other fish were also found in the stream, but little attention was paid to them, inasmuch as interest was centered chiefly in those forms that are commonly associated with normal and healthy conditions that surround oysters. The oyster beds in particular showed an abundance of the life which has been mentioned, indicating quite clearly that conditions under which the oysters exist are both normal and natural. If such were not the case, then the life of the stream, particularly the microscopic life, would be depleted and extinct!

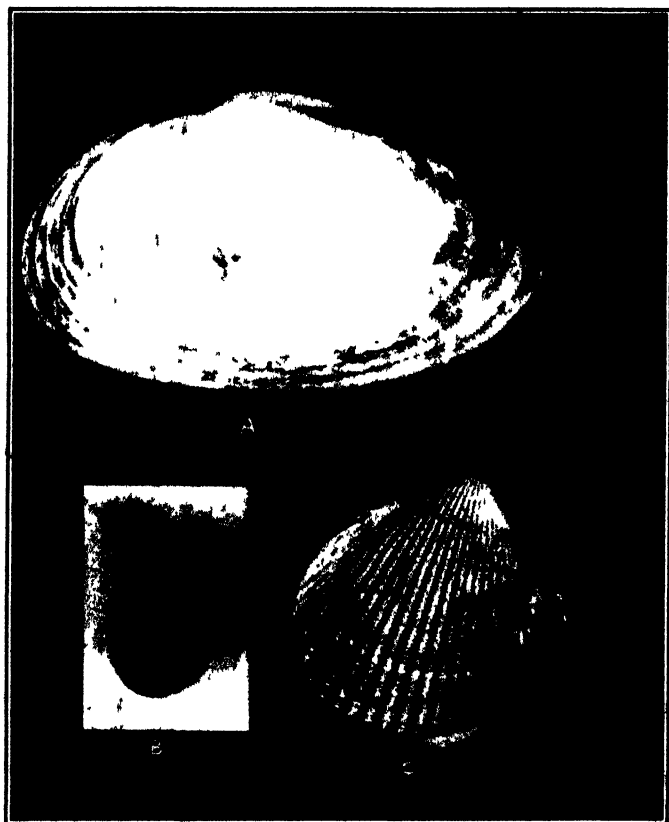


FIG. 8. Common species of molluscs found in Yaquina River. A, *Mya arenaria*; B, *Mytilus edulis*; C, *Cardium corbis*. $\times \frac{1}{2}$.

HISTORY OF OYSTER BEDS

As far as could be ascertained, the natural oyster beds in the Yaquina have existed from time immemorial. Oystermen of the district who have lived in the region for a long time are of the opinion that the first white man to discover the oyster beds was the father of Mr. George Lewis, a fisherman who now resides at Oyster-ville, having been born there about sixty years ago. Even Mr. George Lewis himself can not give the exact date of their discovery. Conservatively estimated, the beds have been worked by the white man at least seventy years, and during this time tremendous quantities of

oysters have been brought up and utilized locally or shipped to distant markets such as Portland and San Francisco. There have really been three distinct periods in the history of the oyster beds of the Yaquina region.

1. *Early Period.* This period, running from about 1860 to 1870, involves the time when the oyster beds were first discovered by the white man, when population in the west was scarce and the only large settlement was that of San Francisco. The old-timers of the Yaquina state that during this period large numbers of schooners came up the Yaquina River and dredged out tremendous quantities of oysters, virtually taking them out by scow loads, and transporting them by boat to the San Francisco markets for consumption. No thought was given at this time to conservation, for no one dreamed that the oyster beds could ever be exhausted.

2. *Middle Period.* This refers to the period when the state of Oregon and private citizens of the state definitely obtained control over the oyster beds. It runs from about 1870 to 1923, and during this time the beds were worked heavily and continuously, and no thought was given to replenishing the diminishing supply. Many of the beds became so depleted that they were virtually exhausted. During the last few years of private control the oystermen encountered considerable difficulty and catches were materially decreased. The important fact to bear in mind is that no attempt was made towards experimentation for the purpose of maintaining or increasing the yield of oysters.

3. *Recent Period.* This dates from the year 1923, when the Oregon Oyster Company took over most of the private beds and leased the state's natural beds. When this concern got control of them they were already in a dangerous state of depletion. Instead of surveying them carefully for purposes of applying measures which would build them up and conserve the fast diminishing supply of oysters, they rather increased the damage by their heavy dredging and tonging operations. Many of the

beds which were in a state of partial depletion were practically wiped out by such methods, and needless to state, this has proven disastrous to the industry.

In going over the history of the oyster beds, it becomes apparent that they have been in a state of depletion for a good many years. The following quotations from official bulletins published by the state of Oregon are extremely significant. In the first biennial report of the state biologist for 1901, Appendix C—"Present Condition of Eastern Oyster Experiment and the Native Oyster Industry," F. L. Washburn, state biologist, says, "Yaquina Bay oystermen get at present \$2.50 to \$2.75 per sack for native oysters, a San Francisco firm having contracted with most of the oystermen for this season's output at the latter figure. The oysters on the native beds are so closely worked now that one-half a sack on a tide is considered fair work, though one sack is sometimes obtained. In the past, the business has been much more profitable than at present. The oysters have dwindled in numbers and in size, owing to a too persistent tonging, together with a lack of foresight on the part of the oystermen. If they could unite in a determination to forbid all tonging for two years or more on certain reserved portions of the natural beds, and persist for a number of years in such a plan, using care with the unmarketable seed, besides taking the best possible precautions along modern lines for catching spat, I believe the industry could be restored to something like what it was ten years ago. But if the present methods continue, I will predict the extinction of the industry before many years."

Two years earlier, in 1899, this same investigator, in an article in *Popular Science Monthly* entitled "Eastern Oyster Culture in Oregon," made somewhat similar significant assertions. He said, "*Ostrea lurida*, the toothsome little native oyster which years ago was so abundant at Yaquina Bay, affording support to many families, has decreased in numbers to such an alarming extent that unless some radical measures are soon taken to prevent,

the native oyster industry of this locality will be a thing of the past."

In the second biennial report of the state biologist under date of 1905, Albert R. Sweetser, then state biologist, in speaking of the eastern oyster experiments in the Yaquina region urges the need for such research, especially for the purpose of obtaining "a species that will multiply and be available to eke out the insufficient supply of natives."

In the fourth biennial report of the state biologist dated 1909, State Biologist Albert R. Sweetser, in his letter of transmittal, makes the following comment on the diminishing yield of oysters. "The experiments with the eastern oysters have shown that it is impossible to make them breed in these waters because of the low temperature, and attention is being turned to native oysters and the possibility of increasing the supply on the natural beds or stocking new localities, the latter being of special interest in Coos Bay, where at present the oyster is extinct though at one time flourishing abundantly. It seems very desirable that the appropriation should be continued that part of it may be used in such experiments." In the same report he also states, "These results suggest other problems. 1, The feasibility of raising spat on a larger scale by some similar method and restocking the beds which are rapidly being depleted."

In December, 1918, Carl D. Shoemaker, then state game warden for Oregon, made the following statements in his 1917-1918 Report to the Board of Fish and Game Commissioners: "The natural restocking of the oyster beds of Oregon has fallen off to such an extent that the industry to-day can hardly be said to be profitable. Yaquina Bay offers the most favorable location for artificial restocking of this excellent food. I would recommend that legislation be enacted giving greater latitude to the Fish and Game Commission, to the end that this work may be better carried on."

In going over the beds and in talking with the oystermen it becomes apparent that practically nothing has

been done in bygone years to conserve the oysters for future generations. The oystermen have been interested exclusively in exploitation for gain, and have done almost nothing to help nature along in replenishing the supply. It must be remembered that the beds of the Yaquina region have been extremely limited. For instance, in the Third and Fourth Annual Reports (1895-96) of the State Fish and Game Protector, Hollister D. McGuire, to Wm. P. Lord, Governor of Oregon, we meet with the following assertion: "The oyster industry of the state at the present time is of small importance, judging from amount and value of the output. The industry of Oregon is confined to Yaquina Bay, at which place there are twelve private oyster beds, staked as provided by law; also, a small area of natural beds from which the supply for renewing the private beds of the oystermen is obtained. The output of the oyster beds at Yaquina Bay from June 15, 1895, to June 15, 1896, amounted to two thousand sacks of one hundred pounds each, the most of which was shipped to the San Francisco market and brought the oystermen \$3 per sack, or a total of \$6,000 for the year." Then again, in the Fifth and Sixth Annual Reports (1897-98) of the State Fish and Game Protector, Hollister D. McGuire, to Governor Wm. P. Lord, of Oregon, the following pertinent statements are made: "The oyster industry of the state is at present confined to Yaquina Bay, and is of small proportions. The native oyster of this bay is noted for its excellent flavor and brings a higher price than the native oysters of Puget Sound and Grays Harbor. From June 15, 1896, to June 15, 1897, there were shipped from Yaquina and consumed locally 1,406 sacks of oysters, which brought the oystermen \$3 per sack, or a total of \$4,218, most of the output going to San Francisco."

It is thus evident that the oyster area has been quite limited and therefore only a limited supply of oysters could be expected annually. Obtaining more than this yield soon upsets the natural balance with the result that

fewer and fewer oysters remain on the beds and so less and less young are annually produced. It is perfectly obvious that such a procedure soon wipes out any natural resource, and this is exactly what has been happening to the oyster beds of the Yaquina region! Conditions would not have been quite so bad if the oystermen had made an intelligent attempt to study the situation for the purpose of finding a remedy which would counterbalance the depletion; but as far as could be determined, almost no scientific work of such a character has been undertaken.

Prior to 1923, the date when the recent period in the history of the oyster beds began, the oystermen operated in a limited way, and in spite of the fact that the beds were already in a state of depletion and also that the oystermen had difficulties such as were pointed out previously by Edmondson, they made a fair catch which netted them a fair return. Since 1923, there has been no let-up and the exploitation of the oyster beds has increased to the extent where many of them have become seriously depleted. In order to increase yields, many of the adult oysters with spat were dredged and tonged up from the natural beds and then distributed either on locations which were devoid of oysters, or into the main channel of the river where there were plantings of eastern oysters from the Atlantic seaboard. Such operations soon prove fatal to oyster beds, for the following reasons. In the first place, it removes large quantities of adult oysters and young growing spat from locations where they are developing in a healthy manner. Secondly, transferring them on to depleted areas in the main channel of the stream is decidedly bad, for the oysters are soon covered with mud and silt to an extent where they are virtually buried. The result is that many of them are either killed off or their normal growth is greatly interfered with. Finally, when mud and silt cover the shells they no longer serve as cultch, for this debris makes it impossible for the free-swimming larvae to come in contact with the clean surfaces of the shells

in order to affix themselves. These findings are in agreement with those of Moore (1897), Stafford (1913, 1915), Churchill (1920) and others who have pointed out repeatedly the necessity of favorable bottom locations for successful oyster culture.

Aside from interfering with the natural oyster beds and lack of proper planting of oysters on suitable bottoms, almost no work has been done by the oystermen in supplying the beds with proper cultch at the right time so that swarming larvae will have an opportunity to attach themselves. As already mentioned, cultch refers to the objects to which larval oysters attach themselves. This may consist of pieces of wood, stones, shells of various molluscs and objects of similar character. It has been found that clean shells of oysters or clams, which have been allowed to remain in the open where they can be washed by the rain and bleached by the sun, afford the cheapest and best means of cultch. At the appropriate time, shortly before the larvae of the oysters are ready to attach themselves, these shells must be distributed over the oyster beds in order that the young oysters have an opportunity of attaching themselves. The best time for this depends on microscopic studies of the organisms in the waters over the oyster beds. When large numbers of oyster larvae make their appearance in the catches then is the best time to distribute the cultch!

Stafford in his work with the native oyster of British Columbia, as reported in the publications of the British Columbia Fisheries Department for 1917, tells us that "The time to put out cultch has been and still is largely judged by the results of previous plantings, *i.e.*, by experience. A sort of customary time—about the last of June or first of July—has been arrived at. But this is not equally good for all places. It may be a little late for places to the south or somewhat early for those of the north. Then again, it is not equally good for all years, for the warm weather of one summer may be con-

siderably earlier than for another. There are other things that may interfere, such as sudden changes of temperature or a heavy fall of rain. It is useful to have in mind some approximately correct time as a reminder that certain preparations should be commenced, but the actual time can not be foretold with accuracy for any considerable period in advance. It has to be determined for each year, and, in fact, for each locality, except where places are near together and under like conditions."

In the Yaquina region very little attention has been paid to the problem of cultch. While some shells have been scattered over the beds in a hit or miss manner no systematic attempts have been made in this direction year in and year out. The oysterman has relied largely on nature to do his work, and as already mentioned, nature has limitations beyond which she refuses to go. Man must then come in and afford help or improvement if he wishes to prevent exhaustion and final extermination. In studying the oyster beds, it soon becomes apparent that the oystermen on the Yaquina have done very little in the past to build up the beds or to conserve the supply of oysters on them. This has been the situation for a good many years, with the result that the oyster beds are greatly depleted and the yield from them has become accordingly diminished. With adequate study, care and the application of stringent measures of conservation many of these areas may be revived and the yield increased accordingly, but this will have to come through careful scientific study and research.

PRESENT CONDITION OF OYSTER BEDS

Numerous visits were made to the oyster beds to determine their condition. On October 2nd and 16th Mr. George Lewis conducted me over his oyster beds at Pool's Slough, located in the heart of the oyster district at Oysterville (station 5, Fig. 1 and Fig. 9). Mr. Lewis and I tonged up large numbers of native Yaquina oysters and these were turned over to me for examination. The

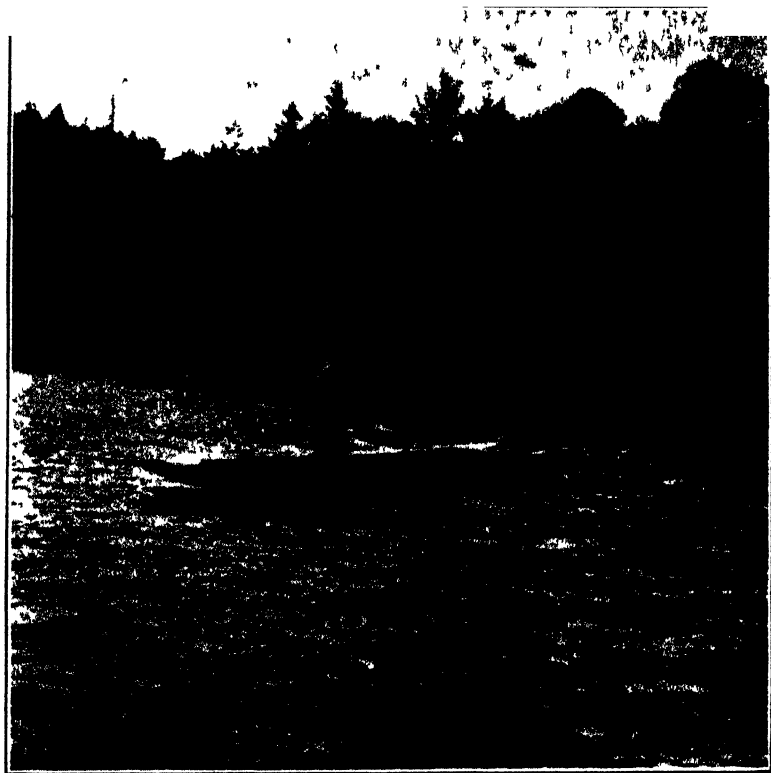


FIG. 9. Oystering in Pool's Slough

oysters were attached to shells of clams, cockles, native and eastern oysters. There were an exceedingly large number of dead shells among them. The majority of the live oysters obtained were two years old, and these were in excellent condition, fat and healthy in appearance. Many were opened on the ground and the mantle cavity was carefully examined. Nothing but sand, mud and fragments of sea weeds were found. They contained exactly the same material as the bottom upon which they were living. Harlan B. Holmes, of the U. S. Bureau of Fisheries, reports similar findings. He states that "in examining the oysters special attention was given to the presence of foreign matter in the mantle cavity. Nothing of any significance was found and the

oysters appeared to be in a normal and healthy condition."

A few cockles and clams were also obtained and these were in fine condition. Included in this catch were also a few specimens of the sea snail (*Thais*), hermit crabs and small Oregon crabs. Some of the oysters had sponges, sea anemones, hydroids, sea mats (bryozoa), sea mosses and algae growing on them. Microscopic examinations of their surfaces later revealed great numbers of diatoms, the natural food which the oysters use. Everything indicated that the bottom of the oyster beds was perfectly normal and in good condition for the existence of living organisms, such as oysters and those forms ordinarily associated with them.

Samples of this bottom débris were bottled and taken to the laboratory for examination, and these were found to consist of sand and mud, rich in diatoms, algal particles, plant fragments, Protozoa and larvae of Crustacea. The diatoms predominated by far. Many of the oysters were also taken to the laboratory for microscopic examination of stomach contents and their food was found to consist of organisms (Fig. 7) similar to those existing in the bottom samples above mentioned.

In spite of all this the contents brought up showed that the oystermen had not given much care to the oyster beds. Spat was in evidence, but this was clustered and crowded on the shells. The oystermen had not gone over the beds for purposes of separating the young oysters so that they would have the opportunity of growing more effectively. Furthermore, the spat seemed to suffer from a lack of good cultch. While shells were present they appeared to have been on the beds a very long time and were covered with large numbers of barnacles, débris or slime, which prevented many spat from attaching themselves. It seemed evident that no systematic efforts had been made by the oystermen to build up or maintain the beds by the logical accumulation of cultch and its distribution over the beds at the proper time.

On other occasions large numbers of oysters were obtained off the natural and planted beds controlled by the Oregon Oyster Company. From the natural beds in the main channel and near the west and southwest shores in the vicinity of station 4, Fig. 1, about two bushels of native oysters were obtained and carefully examined. Many of them were opened directly on the ground and were observed to be in a healthy and normal state. The beds upon which they were located revealed nothing but mud and sand. Bottom dredging in this vicinity brought up only mud and sand. Under the microscope this material was found to be rich in normal oyster food. Laboratory examinations of the stomach contents of the oysters disclosed the typical oyster diet, consisting largely of diatoms and algal fragments. Also, oyster spat of various ages was in evidence here (Fig. 10).



FIG. 10. Spat of native Yaquina oysters. $\times 4$.

In spite of all this, it soon became evident that the beds were in very poor condition, like those in Pool's

Slough. When I remarked about the condition of these oyster beds to Messrs. George Lewis and Tom Christensen, fishermen residing at Oysterville, they responded by saying, "Why shouldn't the beds be in poor shape? They have been neglected and given no care. Furthermore, in recent years they were stripped of most of the oysters by heavy tonging or dredging operations and this has proved ruinous to them."

From the planted beds of the Oregon Oyster Company many eastern oysters were obtained through Mr. Meinert Wachsmuth, one of the owners of the firm residing at Oysterville. The beds where the eastern oysters were planted lie on the shore flats below low water mark, starting a little below Mr. Wachsmuth's home and running half way the distance towards Pool's Slough. The location is along the south shore in the vicinity of station 7, Fig. 1. Fig. 3 indicates the location. It is of interest to note that the beds upon which the eastern oysters were planted at one time were rich in native Yaquina oysters, but because of heavy oystering they have been wiped out almost entirely, so that at present only a very few of the native forms are ever obtained on them.

The eastern oysters which were planted were adults, about three or four years old, with the idea undoubtedly in mind of having them available for market very soon after planting. The Oregon Oyster Company did not bother with seed oysters, which are generally around a year old, because these would have to feed and grow for about three years before they would reach marketable sizes. It is important to bear these facts in mind in order to explain the mortality which has been going on amongst the transplanted adults.

Mr. Wachsmuth brought up over five bushels from his oyster beds, consisting almost exclusively of adult eastern oysters, either alive or dead. Included in this catch were large quantities of black mud, silt and sand, samples of which were obtained for examination. Dredgings in the main channel near these beds gave similar

bottom samples. In the laboratory these materials showed the usual oyster food, such as has already been described. Another interesting feature of this catch from the planted beds was the fact that over half of it consisted of dead eastern oyster shells. The oysters which were alive consisted of a few native Yaquinas which were in excellent shape, and the rest, *i.e.*, the vast majority of them, eastern oysters. These latter could be classified into two groups: (1) healthy, grayish and fat, and (2) sickly, greenish and thin. An examination of both these latter groups, particularly of the second, showed that a large number of the shells had been attacked by the boring sponge found on many of the oyster beds of the Atlantic coast. These shells were riddled with numerous holes and excavations which weakened and destroyed them. The sponge, in its boring operations completely perforates the shell so that the oyster is constantly using up its energy in healing over these places. The result is that the oysters are weakened to such an extent that many of them die. In the burrows of the shells, immediately underneath the outside layers, there were invariably found annelids and other minute organisms, depriving the oysters of food and adding to the damage, thereby hastening their death. Fig. 11 is a photograph of one of the transplanted adult eastern oysters from these beds, showing the condition.

On the shore beside Mr. Wachsmuth's home there was a large pile of eastern oyster shells and when these were examined it was found that the shells showed distinct traces of having had live oysters in them and opened recently. Furthermore, nearly half of the pile consisted of shells which were riddled by the excavations caused by the boring sponge. Undoubtedly this disease was brought with the oysters from the east, for, as has been pointed out, many of the areas on the Atlantic coast have suffered from the ailment. That this is not a disease of the Yaquina oysters is borne out by the fact that in not a single instance was it discovered on any of the native oyster shells examined.



FIG. 11. Eastern oyster showing effects of boring sponge. Young spat of native oyster near lower margin. $\times \frac{1}{2}$.

Many of the live oysters were opened and examined on the ground. Careful search of the mantle and gill cavity revealed sand and mud particles, similar to the bottom on which the oysters were living. About a bushel of the eastern oysters and many of the native Yaquinas were taken to the laboratory for study. Examinations under the microscope of stomach contents revealed natural oyster food—diatoms, algae, Protozoa and the like similar to the organisms dwelling on the surface and bottom areas where the oysters were living.

While spat was scarce, nevertheless it was found on quite a number of shells (Fig. 12), even settling on shells



FIG. 12. Eastern oyster with spat of native Yaquina oysters. $\times \frac{1}{2}$.

which were riddled with cavities produced by the boring sponge. Of course, this spat consisted of the native Yaquina oyster, *Ostrea lurida*, and not of the transplanted eastern oyster, *Ostrea virginica*, which apparently does not reproduce in the Yaquina location.

Concerning the condition of the eastern oysters, there are numerous reasons which account for it.

(1) From all the information available, the oysters, when they arrived from the east, were in exceedingly poor shape. Fishermen in the vicinity stated that about half of the oysters shipped from the east arrived either dead or in a dying condition. Many of the live ones had

their beaks broken off, exposing the soft inner parts, making it rather easy for enemies to enter and destroy them.

(2) Large numbers of oysters shipped were undoubtedly parasitized by the boring sponge so that this disease was transplanted to the Yaquina from the eastern locality where the oysters developed. Inasmuch as this parasite weakens, thins and ultimately kills oysters, there is not a question of a doubt that many of the oysters which were planted soon died on their new location in the Yaquina River.

(3) The eastern oysters which were planted were adults, about three or four years old. In practice it has been found that the best results are obtained by planting seed oysters, those which are small and around one year old. The logic of this is quite obvious. The adult oysters had accustomed themselves to a certain environment on the Atlantic coast, and then they were shipped nearly 3,000 miles to their Pacific location on the Yaquina River, where they had to meet different conditions and acclimate themselves to a virtually new environment. Young oysters are quite plastic and can adjust themselves very readily to such changes. On the other hand, the adults have become more or less fixed and are not able to orient themselves nearly as readily. The same parallel may be drawn between the adjustments of children and adults among humans. In spite of all this, some of the eastern oysters did adapt themselves to their new locations and have grown into fat and healthy individuals. Many others, however, could not so adjust themselves and soon died. A large number of those which were weakened in transit or arrived in a diseased state also succumbed. The sum total of all this has been that many dead oysters are found at the present time on the planted beds.

That the adult eastern oysters seem to succumb more readily in the Yaquina region than the young ones is a matter of official record. The first shipments of eastern oysters to the Yaquina region came from the U. S. Bureau of Fisheries between the years 1896 and 1899,

and they consisted of 32 barrels of seed oysters. They were planted near Oysterville and for a few years seemed to be doing exceptionally well. However, in 1904, State Biologist Albert R. Sweetser, in his second biennial report, tells us in reviewing eastern oyster experiments that "some of the oysters have reached the age of six or seven years, which some regard as the limit of life, though others maintain that they can continue to grow and fatten for a number of years more. We are finding many empty shells and it would seem that they are dying of old age."

Then again, in the third biennial report for 1907, State Biologist Albert R. Sweetser, in commenting on the eastern oyster experiments, remarks, "It was decided to transfer the oysters to new beds in search of more favorable environment, and also to protect them from the injury by the dragnets of the fishermen. . . . They were situated on the north shore of Yaquina Bay near the railroad station of Winant. Mr. George King offered the use of one of his private beds on the south side and almost opposite. . . . In making the transfer many empty shells and dead oysters were found."

(4) The eastern oysters were planted in a bad region, which received large quantities of silt, mud and sand, burying the oysters, killing many of them off and preventing spat from settling on their shells. The question of where the best place is for planting the eastern oysters in the Yaquina River has never, to my knowledge, been accurately determined. As has been pointed out, the area in which they were planted does not seem to be the best section. It appears that no observations or experiments have been made to determine the locations most suitable for obtaining the best results with eastern oysters. The procedure has been largely a hit or miss proposition, and unquestionably this has accounted for the results obtained.

That eastern oysters will fatten and grow large in the Yaquina River is evident from the fact that quite a number of the transplanted forms have established them-

selves and have grown into vigorous and healthy individuals (Figs. 5 and 12). With careful study of the environment there is no reason why it could not be determined where the raising of these forms could be accomplished with greatest success.

However, it seems fairly certain that these eastern oysters do not produce spat that will perpetuate the species. Numerous experiments have been tried in the early days to induce eastern oysters to become established and reproduce themselves in the Yaquina, but ultimately they were all given up as failures. In 1908, State Biologist Albert R. Sweetser, in the letter of transmittal which accompanied his fourth biennial report to Governor Chamberlain, wrote, "The experiments with the eastern oysters have shown that it is impossible to make them breed in these waters because of the low temperature." A similar state of affairs has been found to exist in practically all other oyster areas along the Pacific coast.

Concerning the question as to why large numbers of spat of the native Yaquina oysters are not found on the shells of the transplanted eastern ones, the reasons unquestionably are as follows: In the first place, the eastern oysters were planted on a bed where almost no native oysters remained. The latter were tonged or dredged up in bygone years without allowing the beds to repopulate themselves. Therefore, at the present time, there are hardly any of the breeding adults left in the region and therefore very few larvae, if any, are available for setting. In the second place, the eastern oysters were in a location where large quantities of mud and silt cover them, with the result that larvae in this region have found it difficult to encounter clean surfaces of shells to which to attach themselves. As has already been mentioned previously, spat has been encountered on quite a number of the eastern oysters indicating clearly that if clean shells were placed in the water at the right time, in regions where native Yaquina oyster larvae abound, a good crop would undoubtedly result.

Finally, after a careful consideration of all of the evidence the chief reason for the depletion of the Yaquina oyster beds, aside from the natural obstacles mentioned above and the limited area, has been the devastating practice of exploitation which has been going on, without adequate and scientific measures of conservation to maintain the supply. This question has already been alluded to and it will be necessary to repeat only the salient facts. The oyster beds have been neglected; many natural beds have been ruined by dredging and tonging operations; the best regions for spat, such as sloughs, have not been utilized or developed; no scientific work has been done to determine time of spawning; no adequate provisions have been made for cultch; no attempts have been made to overcome natural obstacles presented by the Yaquina River, such as mud, silt and sand distributed by the tides. In a word, the greatest enemy to the oyster beds of the Yaquina region has been the oysterman and his unscientific methods of exploitation without adequate measures of conservation. As will be recalled, State Biologist Washburn sounded the alarm with regard to the Yaquina oyster beds as far back as thirty-two years ago!

In recent years, the oystermen instead of attempting to improve conditions have simply added to the ruin by their unscientific methods and practices. After all is said and done, it must not be forgotten that where a limited producing region is available, only a limited natural yield is possible. Stretching it beyond this point is soon disastrous, unless intelligent care and adequate conservation measures are applied to maintain it. Even then, nature often refuses to bring forth the abundance expected, with the result that exhaustion or extinction invariably follows. This is what has happened to most of the oyster producing regions on the Yaquina River!

SUMMARY

1. The Yaquina oyster beds of Oregon are located in that portion of the Yaquina River lying mainly in the

vicinity of Oysterville, beginning approximately six miles below the town of Toledo and extending for nearly three miles in the direction towards Newport.

2. The oyster beds are limited in area, comprising no more than 135 acres at the most. The large bulk of the oysters consists of the native Pacific species, *Ostrea lurida* Carpenter. There have also been numerous plantings of the Atlantic oyster, *Ostrea virginica* Gmelin, but with few exceptions these have not acclimated themselves to their new locations in the Yaquina region.

3. The Yaquina River is an exceedingly narrow and shallow stream carrying a large amount of sediment and débris. Inasmuch as this suspended material settles on the oyster beds, it becomes absolutely necessary for the oystermen to keep constant watch over their beds and give them the very best of care and cultivation.

4. The salinity and food in the vicinity of the oyster beds are adequate for the proper development of oysters. Moreover, there is an abundance of natural life in the Yaquina River, indicating that stream conditions are natural and wholesome not only for oysters, but also for those forms invariably associated with them.

5. Untreated human sewage enters the Yaquina River from the various towns located along its banks and this is then carried by the tides back and forth over the oyster beds, making it possible for the oysters to become polluted with dangerous disease producing organisms.

6. The oyster beds have been steadily and consistently worked for the last seventy years. A large quantity of oysters has been removed and little attention has been paid to conservation measures looking towards the building up and maintenance of the beds. The inevitable result has been that many of the beds have become exhausted and the yield of oysters has diminished to the extent where the remaining beds are threatened with extinction.

7. Carefully planned surveys and experiments will have to be initiated to maintain, replenish and repopu-

late the Yaquina oyster beds if they are to be conserved for future generations.

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SHORTER ARTICLES AND DISCUSSION

ORDER OF EMBRYONIC SEGREGATION IN INTER-MEDIATE APHIDS NOT REVERSED BY LOW TEMPERATURE¹

IN a study of the composition of intermediates between gamic and parthenogenetic aphids (Shull 1930b) it was found that a change from parthenogenetic to gamic individuals produced the same type of intermediates as did change from gamic to parthenogenetic, and that the order in which the distinguishing marks became altered was reversed in the two directions of change. When the offspring were changed from gamic to parthenogenetic, by removing their winged parents from low to high temperature, the earliest offspring to show an effect of this change possessed modified antennae and tibiae, and only in offspring born later were the reproductive system and the color of the body altered. In these modifications of the reproductive system, the earliest offspring lost their colleterial glands and seminal receptacle, while later ones exhibited various changes in the ovarioles. The order of change in successive offspring is thus antennae and tibiae, then colleterial glands and seminal receptacle, then ovarioles, with body color changing along with the latter two changes. When the offspring were changed from parthenogenetic to gamic, by changing the winged parents from high to low temperature, the earliest intermediate offspring showed modifications of the reproductive system and body color, while only later offspring showed changes in the antennae and tibiae. The intermediate reproductive systems were too few in this latter transition to fix certainly the relative time of change of colleterial glands and ovarioles, but aside from this the order of change in successive offspring was the reverse of the order of change when gamic offspring were being gradually converted into parthenogenetic.

The nature of these intermediates is of considerable genetic interest partly because of the probability that they are produced under the same fundamental conditions as are intersexes. Goldschmidt explains intersexes as due to a dichotomy of embryonic

¹ Contribution from the Zoölogical Laboratory of the University of Michigan.

segregation (embryonic determination) plus a turning point in physiological properties affecting development. The dichotomy of segregation entails the determination of different structures successively instead of simultaneously, a condition well established in a number of vertebrate animals. The turning point, so far as concerns intersexes, is the time at which, owing to a rise in the level of certain processes, developmental influences change from female-determining to male-determining or the reverse. Structures segregated (determined) before the turning point are like those of one sex, those segregated after the turning point are like those of the other sex. Goldschmidt has summarized his theory of intersexes in a recent review (1931) in which his earlier papers and other pertinent literature are cited.

If Goldschmidt's theory is applicable to intermediate aphids, those intermediates which are produced during a change from parthenogenetic to gamic should possess different characters from those produced during a change from gamic to parthenogenetic. The order in which the structures change should be the same in both, but since in one of the changes a given organ should appear while in the opposite change that organ should be lost, the composition of the intermediates produced should be quite different in the two cases. As stated above, these conditions did not exist in the intermediates obtained in my earlier work; the order of change was apparently reversed, while the composition of the intermediates was the same regardless of the direction of change.

Three possible explanations of this result were discussed but none of them adopted in preference to the others for want of decisive evidence. Two of them permitted the adoption of Goldschmidt's theory, the third was opposed to it. These explanations were briefly as follows: (1) that the intermediates which were born during the transition from parthenogenetic to gamic were not produced then but had been produced earlier during a change from gamic to parthenogenetic (it had been impossible to get a transition from parthenogenetic to gamic except in families that had first been changed from gamic to parthenogenetic by high temperature), and were necessarily of the same composition; (2) that the low temperature which induced the change from parthenogenetic to gamic offspring had, by a differential effect on the rates of development of different structures, reversed the order of segregation of antennae and

tibiae as compared with the reproductive system; and (3) that the nature of intermediates is due, not to the order in which their structures are embryonically segregated, but to the level at which some physiological property exists regardless of the direction from which that level has been approached. The first of these explanations permits the adoption of Goldschmidt's theory but does not necessarily favor it; the second directly recognizes that theory and is built upon it; the third is opposed to Goldschmidt's theory.

Some evidence has now been obtained which tends to show that the second of these explanations is incorrect. It consists of a number of winged aphids, of the same species as was previously used (*Macrosiphum solanifolii*), which possessed thickened hind tibiae. Gamic females, which normally possess thickened hind tibiae, are regularly wingless. Winged individuals with thickened tibiae are therefore one form of gamic-parthenogenetic intermediate. Two females of this kind had been previously obtained, several years apart, and their significance appreciated; but they were too few to form the basis of any valid conclusion. Now, however, 34 more of this kind have been produced, in a single experiment in which several hundred intermediates of various types appeared. Within this single experiment it would seem to be particularly safe to compare the intermediates with one another.

While all of these intermediates are being used in a comprehensive study of intermediates in general, it is with respect to only one feature of them that the present paper deals, namely, the relative thickness of the hind tibiae in winged and wingless intermediates. The tibiae of the winged intermediates are thinner than those of the wingless ones. The method of recording thickness of the hind tibiae, as described in an earlier paper (Shull 1930a), is to rate the thick tibiae of the gamic females at 0, the thin tibiae of the parthenogenetic females at 4, and the intermediates at regular intervals (1, 2, 3) between these extremes. A comparison is here made only between intermediate females appearing in the same experiment. Since all the winged females with somewhat thick hind tibiae had reproductive systems that also were partly parthenogenetic and partly gamic, they are compared only with wingless females whose reproductive systems are in some manner a mixture of the two types. In this comparison it is found that the mean thickness of the

hind tibiae of the winged gamic-parthenogenetic intermediates was $3.14 \pm .07$, while that of the wingless gamic-parthenogenetic intermediates was $.74 \pm .09$. A more restricted comparison may be made by selecting only those possessing the same type of intermediate reproductive system. Of the winged ones all but two had parthenogenetic ovarioles but colleterial glands and seminal receptacles. The 32 possessing such reproductive systems had hind tibiae averaging $3.10 \pm .07$, while the 29 wingless intermediates with the same type of reproductive system had hind tibiae whose thickness was $.82 \pm .15$.

It is clear from the above that possession of wings in a gamic-parthenogenetic intermediate carries with it a thinning of the hind tibiae. Without implying that any such physiological relation is involved, it is as if something was consumed in producing wings which would otherwise have gone into thickened tibiae. Now, it was shown in earlier experiments (Shull 1928) that whether a female is to be winged or not is not decided (at least so far as light and darkness are concerned) until the last 48 hours (approximately) before birth, or probably even within the last 24 hours. Presumably, therefore, from the correlation described above, the thickness of the tibiae is likewise not determined until shortly before birth. Since it has been found impossible to alter any of the aphids by applying to them after birth the treatments that have been shown to be efficacious before birth, it appears almost certain that the wings and the nature of the hind tibiae are among the last things to be segregated in the course of embryonic development.

What does this fact signify with regard to the three possible explanations described above as accounting for the similarity of intermediates produced during changes in opposite directions? All of the winged gamic-parthenogenetic females described in this paper were the offspring of parents reared at low temperature until just before these intermediates were born. If low temperature has a differential effect on the rate of development of tibiae as compared with reproductive systems, such as to reverse the order of their determinations, it should have done so in these winged intermediates. The second of the three explanations just referred to requires that, at low temperature, the nature of the tibiae be determined earlier than that of the reproductive system. The correlation between wings and tibiae shows that the tibiae are determined quite late at low tempera-

ture, hence it is scarcely possible that the reproductive system is determined still later.

It appears practically certain, therefore, that the second possible explanation of my earlier experiments must be abandoned. The first and third, one of them consistent with Goldschmidt's theory of intersexes, the other not, remain as possibilities. It is hoped that the more complete study of intermediates which is in progress will throw light on the relative values of these two explanations.

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CHROMOSOME NUMBERS IN *PHLOX*

ANTECEDENT to a genetic investigation on the genus *Phlox*, chromosome counts were made of twenty-five varieties distributed among thirteen species. Kelly (1920) reported that the chromosome number in *Phlox drummondii* is fourteen for the cells of the root-tip. Kelly and Wahl, in a later report (1928) state that "many chromosome counts from pollen mother cells of different species of *phlox* by the Belling method have indicated that the number of chromosomes in the somatic cells of *P. drummondii*, *P. subulata*, *P. stolonifera*, *P. divaricata*, *P. pilosa*, *P. glaberrima*, *P. ovata*, *P. maculata* and *P. paniculata* is 14."

All the chromosome counts in the present investigation were made from bud material. The haploid counts were from pollen mother cells and the diploid counts were usually from the somatic tissue of the ovary. This latter tissue abounds with division figures in most species and it is not difficult to find stages in which the chromosomes may be counted. In most species division figures were equally numerous regardless of the time of day at which fixation occurred. The buds were fixed equally well in Bouin's fluid and in Allen's modification of Bouin's. Carnoy's fluid gave poor results. Following fixation the buds were dehydrated, cleared in xylol, and imbedded in paraffin. Sections cut at ten microns proved best for the study. The sections were stained in Haidenhain's iron-alum haematoxylin.

The chromosomes of all the species observed were relatively very large.

The following table gives the chromosome numbers and the source of the plants from which material was obtained for this study. The nomenclature is in most cases that of "Standardized Plant Names."

		N	2N
<i>P. adsurgens</i>	(Oregon Gardens)	—	20 +
<i>P. amoena</i> Sims (<i>P. procumbens</i>)	(Dreer and Oregon Gardens)	6-8	14
<i>P. arendsii</i> varieties			
Charlotte	(Bobbink & Atkins)	—	14
Hanna	(" " ")	—	14
Hilda	(" " ")	7	14
<i>P. argillacea</i> Clute & Ferris	(Oregon Gardens)	7	14
<i>P. diffusa</i>	(Oregon Gardens)	—	28
<i>P. dwaricata</i> L. (<i>P. canadensis</i> Sweet)		7	14
<i>P. douglasii</i>	(Oregon Gardens)	—	14
<i>P. drummondii</i> Hook. (several types including Star)		7	14
<i>P. glaberrima suffruticosa</i> var.			
Miss Lingard	(Valley View)	7	—
<i>P. maculata</i> L. var.			
Alpha	(Bobbink & Atkins)	7	14
<i>P. ovata</i> L. (<i>P. carolina</i> L.)	(Dreer)	7	14
<i>P. paniculata</i> L. varieties			
Eclairleur		—	14
Elizabeth Campbell		7	14
Mrs. Jenkins (Independence)		—	14
Mrs. Milly van Hoboken		—	14
Pantheon		7	14
Rheinlander		—	14
R. P. Struthers		7	14
Siebold		7	14

Sunset	—	14
Thor	—	14
(various commercial seed houses)		
<i>P. stolonifera</i> Sims (<i>P. reptans</i> Michx.) (Oregon Gardens)	—	14
<i>P. subulata</i> L. varieties		
<i>rosea</i>	(Valley View)	7 14
<i>hlacina</i>	(" ")	— 14

Only one plant of *Phlox adsurgens* was available. The chromosomes could not be counted in the pollen mother cells of any of the few buds which the plant produced in 1931. This species, unlike most of the others dealt with, does not show a profusion of division figures at all times of the day. In the several buds sectioned there were no good somatic figures. In several figures at least twenty chromosomes were counted. In these several chromosomes were so clumped as to make exact counting impossible. It appears probable that the chromosome number in this species may be twenty-one, in which case there is a possibility of its being a hybrid between a twenty-eight and a fourteen chromosome type, both of which are native to the same general region.

The pollen mother cells of *Phlox amoena* contain six, seven, and eight chromosomes. This appears to be due to irregular haplois. There are from two to eight "early" chromosomes on practically every heterotypic spindle. At anaphase the chromosomes are scattered on the spindle from the equator to the poles. On some of these spindles seven chromosomes go to each pole. On others six chromosomes go to one pole and eight to the other. According to Bailey (1930) *Phlox amoena* is rarely cultivated—the plant grown under this name being *P. procumbens*. This is a supposed hybrid between *P. stolonifera* and *P. subulata*. The *P. amoena* plants which furnished the material used in this study are especially vigorous for *Phlox* plants, and spread very rapidly by runners. This—together with the irregularity of the chromosome behavior at haplois—lends color to the supposition that the plants dealt with are a hybrid species.

In all the buds of *Phlox divaricata* var. *alba grandiflora* that have been examined the anthers are aborted. Apparently no good pollen is ever formed. Also the ovaries are aborted in over half the buds.

The ovaries of *Phlox stellaria* appear almost always to be aborted. One bud out of twenty-seven examined was found to have a developed ovary and in this the ovules were aborted.

Normal appearing pollen is formed. According to Bailey (1930) "the plant cultivated under this name is apparently a hybrid between *P. bifida* and *P. subulata*." I was unable to count the chromosomes of *P. stellaria*.

During the summer of 1930 attempts were made to cross *Phlox paniculata* reciprocally with *Phlox drummondii*. Approximately sixty crosses were made each way. The crosses in which *P. drummondii* was used as the female parent resulted in three weak-appearing sets. These matured into capsules devoid of seed. No sets were secured from the reciprocal.

In the spring of 1931 attempts were made to cross *Phlox divaricata* reciprocally with *Phlox drummondii*. Forty crosses were made using the former as the female parent. Twenty-six sets resulted. From these twenty-three normal appearing seed were secured. Many of the capsules contained only one seed, or no seed, or seeds that were shriveled. No sets were obtained from thirty-four reciprocal crosses.

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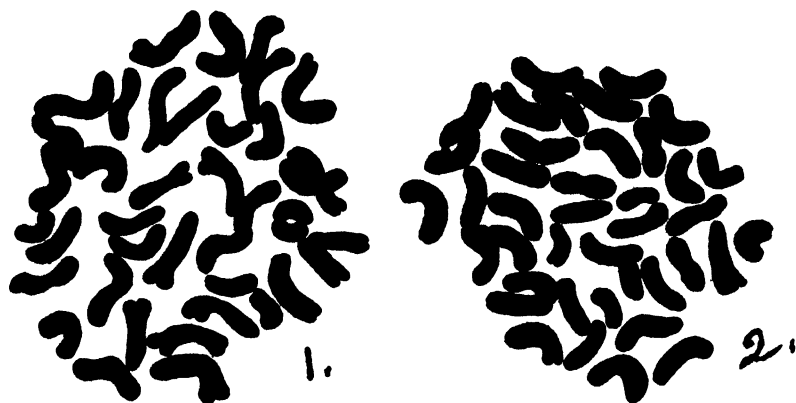
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CHROMOSOME NUMBER IN SPECIES OF PEANUT, *ARACHIS*

PRIOR to a genetic investigation dealing with growth habit, testa color, and size and number of seeds per pod, the somatic chromosomes of six varieties of the commercial peanut, *Arachis hypogaea*, and a strain of the wild Brazilian species, *Arachis nambyquarae*, were counted. Though a study of the meiotic processes and haploid chromosomes is at the present time in progress, this note is merely intended to report the chromosome numbers encountered in root tip cells.

Commercial seed houses furnished the source of the *A. hypogaea* material. The *A. nambyquarae* seed was procured

through the kindness of the United States Department of Agriculture. The seeds of both species were germinated successfully within three days on moist filter-paper. The root tips were favorably fixed in Bouin's fluid and, by the usual method, imbedded in paraffin. Sections were cut $10\ \mu$ in thickness and stained with Haidenhain's iron-alum-haematoxylin.



The chromosomes of all the forms studied, though small and numerous, are well spaced and practically in a single plane at metaphase. The study and counting of them can be done, therefore, with no great difficulty and with little chance for error. The following table gives the diploid chromosome numbers and the specific source of the seed used.

<i>A. nambyquarae</i> (U. S. D. A.)	40
<i>A. hypogaea</i>	
California Long Red (Stumpp and Walter)	40
Improved Virginia (T. W. Wood and Sons)	40
Jumbo (Williams)	40
Spanish (T. W. Wood and Sons)	40
Valencia (T. W. Wood and Sons)	40
Virginia Bunch (T. W. Wood and Sons)	40

The accompanying figures (1. *A. nambyquarae*, 2. *A. hypogaea* var. Spanish), showing the size and general appearance of the chromosomes, were drawn at table level with the aid of an Abbe camera lucida, under a Leitz objective, 2 mm. N. A. 1.32, and a Zeiss compensating ocular 30 $\times$. The magnification is approximately 4500 $\times$.

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GRASS IN A TURTLE EGG

IN preparing embryos of turtles in late stages of development for embryological study at the Beaufort Laboratory, an egg was opened which had a rather well-developed stem of grass inside



FIG. 1. Ventral view showing grass *in situ*.

the shell. There was no apparent opening in the shell through which the grass might have entered and the writer is at loss to explain its presence. Fig. 1 is a photograph of the living embryo



FIG. 2. A. Grass after growth. B. Ventral view of turtle, grass and membranes removed.

and grass in the position in which they occurred within the egg. It is to be noted that the embryo is about ready to hatch.

The grass was removed and placed in a Petri dish with moistened filter-paper. Under these conditions the grass grew for several days. Fig. 2 is a photograph of the grass and the turtle as they appeared when the grass was finally preserved; preservation being necessary on account of a fungus infection.

The grass has been identified by Dr. H. L. Blomquist, of Duke University, as *Spartina patens*, salt marsh grass.

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THE OLDEST MOTH

COCKERELL and LeVeque have just described (AM. NAT.: LXV, 354-355, Fig. 1) a moth under the name of *Chionaemopsis*

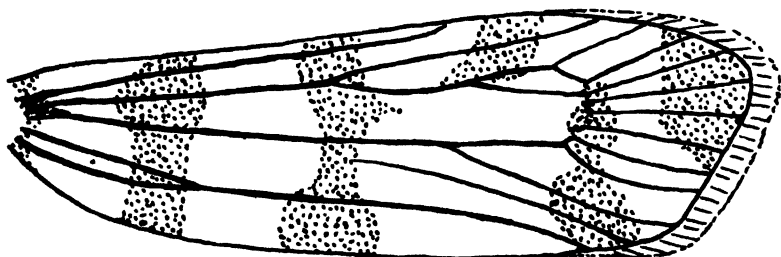


FIG. 1

quadrifasciatus. May I suggest that this is very possibly a species of the modern genus *Atteva* or its immediate ancestor.

On comparing the North American *Atteva aurea* (Fig. 1) with the fossil (Fig. 2) it is seen that the copper pattern cor-

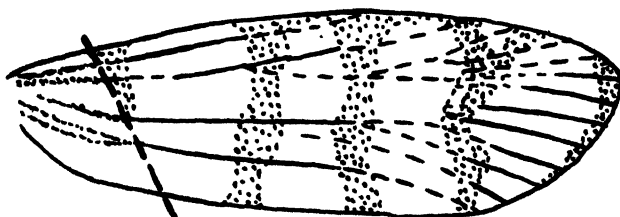


FIG. 2

responds with the dark pattern of the fossil, save that the two postmedial bands are complete in the latter, and that the vena-

tion so far as distinct is of the same type, but the broader wing suggests a transition to *Lactura* or *Urodys*. Cockerell and LeVeque's restoration of the venation can hardly be taken as final, as it would exclude the form from the Lepidoptera. The restoration dotted in on Fig. 2 fits with the traces visible on the half-tone figure, and would make a normal Yponomeutid.

If this form is really an *Atteva*, it is of great interest, as the genus is one of those now represented by well-marked subgroups in the tropics of the two hemispheres, and in addition appears to be a connecting link between the Yponomeutidae and the Pyralids.

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THE MATURATION MITOSES IN CERTAIN PAEDOGENETIC PARASITES

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IN the course of reviewing the material for a general work on evolutionary processes in relation to cytology it has been necessary to consider the reduction or maturation division in a number of animal forms. This procedure was essential because of our lack of information in this important field, especially of a comparative nature. An important theme in this connection is the cytology of parthenogenesis. On the plant side the situation is clear and throughout, in cases of parthenogenesis, an extremely abnormal reduction division is found, presenting a detailed resemblance to the maturation mitoses of known hybrids. It has accordingly been very generally admitted in recent years on the botanical side, particularly for the higher plants, that parthenogenesis is intimately related to previous hybridization. In the case of animals the most important general situation is presented by those forms which are at once bisexual or hermaphrodite and at the same time parthenogenetic. Unfortunately, this group on the animal side is a very restricted one, since animals in general are unisexual. The forms most readily available in the present connection are the flat worms and such nematodes as are parthenogenetic. On account of lack of material the latter group has not been as yet investigated, but the present contribution will deal with the reduction division in tapeworms and flukes so far as it is illustrated in the

material already examined. It is important that these forms should be reinvestigated in the light of our greatly increased knowledge of the cytology of reproduction.

It will be convenient to begin with the common liver fluke, *Fasciola* (Distomum) *hepatica*. This is a form easily available on account of the fact that it is very commonly present in the liver of the domestic sheep. Various methods of preservation have been tried in this connection as the animal, as is common with parasites of this sort, is difficult to preserve adequately. Bouin and other formalin fluids have been used with indifferent results. The best preservation was obtained throughout with Carnoy's formula, 6 alcohol, 3 chloroform, and 1 glacial acetic acid. At first the worms were flattened before being treated with the reagent, but it was found that it was better to immerse them in the preservative and flatten out afterwards. After the material was washed in several changes of strong alcohol it was transferred to equal parts of alcohol and glycerine in which it was left for a few hours. Subsequently the animals were laid down on stiff pieces of cardboard and an abundance of 6 per cent. nitrocellulose was dropped over them. After the nitrocellulose had dried slightly, a piece of heavy paraffined paper of the same size as the cardboard was laid over the animal, then a glass slide on which were placed lead weights to flatten the creature. After the nitrocellulose had set, holding the object firmly to the card, the paraffined paper was wrapped around in two directions with fine white thread, number 50 or 60 gauge. The objects were then dropped into strong alcohol and afterwards pricked with a fine needle, No. 12, mounted in a cork. The pricking is for the purpose of allowing perfect penetration of the nitrocellulose and is essential for obtaining the very thin sections which are necessary. After the worms have been pricked they are put into absolute alcohol and carefully pumped with an efficient air-pump. The ordinary water-pump is not powerful enough for satisfactory results and an electric pump

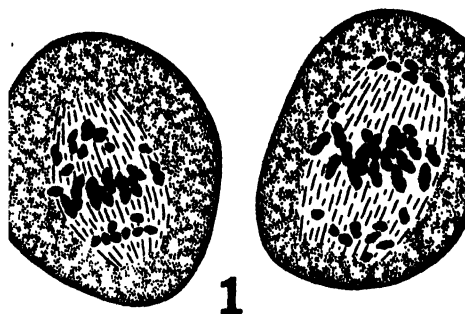
must be used for good results. The material is then run up on the cards in nitrocellulose and embedded after the manner described recently by the present author.¹ After embedding, the sections were made with a sliding microtome and these should be 5 micra or thinner. The most satisfactory stain was Heidenhain's iron haematoxylin. Counter-staining was not found advantageous, as it tends to obscure the details of mitosis. The figures are fairly large in this form and have already been the subject of investigation in recent years by Schellenberg.² Just as in the famous case of *Drosophila melanogaster*, however, his investigations lacked the background of recent developments in the general cytology of meiosis, particularly in plants. The tendency in the past has been to disregard abnormalities in the reduction division and to search for figures which were normal in character. This tendency was quite correct before the cytology of known hybrids and variable species had become as familiar as it is at the present time. It may be stated in a general way that there are many abnormalities in the meiosis of *Fasciola hepatica*. There is also a great deal of sterility, many of the clusters of mother cells breaking down in the course of development. Both the irregularities and the sterility found here are of the type characteristic of known hybrids.

Fig. 1 shows two typical primary spermatocytes of the species under discussion in the metaphase. It can readily be seen that, in the two cells figured, a number of bivalent chromosomes are clustered more or less regularly at the equator of the spindle, whilst towards the poles lie a considerable number of univalents. This mode of division is extremely abnormal and exactly duplicates that found in known hybrids.

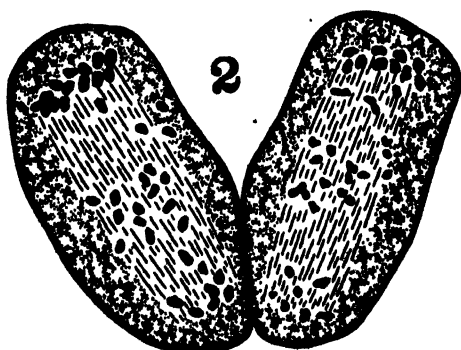
Fig. 2 shows the anaphase in two elongated cells which converge. The plane of section has removed some of the

¹ "Technical Contributions," *Botanical Gazette*, lxxxvi, 4, December, 1928.

² *Arch. f. Zellf.* 6: 443-484, pls. 24-36.

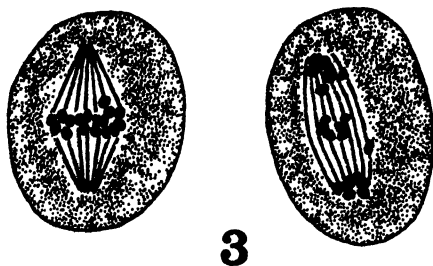


chromosomes at the lower ends of the two spindles. In the upper region numerous univalent chromosomes are present. Additional univalents are scattered on the spindle in the region between the poles. Here again we have the type of anaphase which is characteristic of hybrids and variable species.



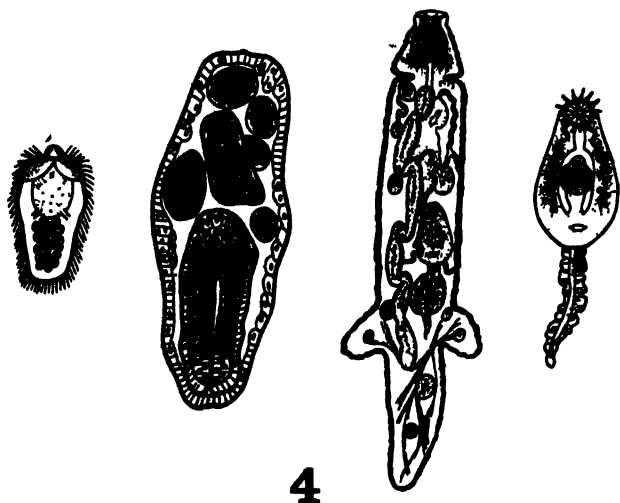
The division of the secondary spermatocytes is much more regular and in the metaphase it is very often difficult to catch any lagging chromosomes. These are more commonly seen in the anaphase, as is shown in Fig. 3.

It will be seen from the above that in many cases the reduction division in the common liver fluke is extremely abnormal and presents those features which are characteristic of hybrids. It has been objected that occasional abnormalities are of slight importance. To this it can be replied that in known hybrids there is a considerable variety of variability in the meiotic mitoses. Some of these are quite normal, whilst others present varying de-



degrees of abnormality. Often in hybrids it becomes necessary to search for the abnormal meiotic features which may be present in such forms. The present writer has pointed out that, however irregular the meiotic divisions may be, in hybrid forms the somatic mitoses are in general extremely regular. This general situation is of course important in connection with the known greater stability of forms of somatic origin.

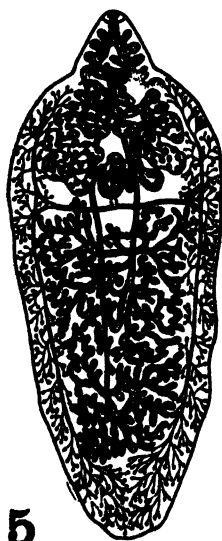
It will be well at this stage to refer in some detail to the life history of the common liver fluke, *Fasciola hepatica*. The eggs, on reaching water, develop into a ciliated embryo (Fig. 4, first from left) which contains a rudimentary ovary internally. These ciliated forms swim about in water or make their way over the surface of vegetation bordering water, whence they penetrate into the bodies of snails. They are, for example, common in our larger Limnaeas. Here the ciliated form develops into a so-called sporocyst (Fig. 4, second from left). From its rudimentary ovary develop embryos parthenogenetically. Since the mother is immature the parthenogenesis in this case comes under the heading of paedogenesis or infantile reproduction. The embryos formed inside the sporocysts escape and in general give rise to a somewhat higher type known as redia (Fig. 4, third from left) which possesses, contrary to the sporocyst, a sucker and a rudimentary intestine. Inside the redias are produced tailed forms with hooks and suckers known as Cercaria (Fig. 4, fourth from left). These, in contrast to the preceding phases, are quite active and escape from the snail, becoming encysted on grasses, etc.



4

Sheep eating these grasses absorb the encysted Cercariae, which develop into the completely sexual stage known as the liver fluke, *Fasciola hepatica*. This condition of het-

erogenesis is extremely common in parthenogenetic animals, being exemplified by many species of insects, such as Aphids, Hymenoptera, etc. The cytology of these forms is being reinvestigated in the light of more recent developments, and an account of it will be published in a subsequent article. It may be stated preliminarily that the results obtained entirely correspond with those found in parthenogenetic plants.

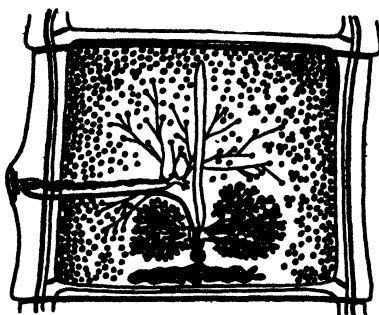


5

Fig. 5 shows the mature animal of the common liver fluke, *Fasciola hepatica*. The only details of structure which need be emphasized in the present connection are the organs of reproduction. In the upper part of the figure is shown the much convoluted ovarian system containing the young and developing eggs. Mainly in the lower part of the figure and in the

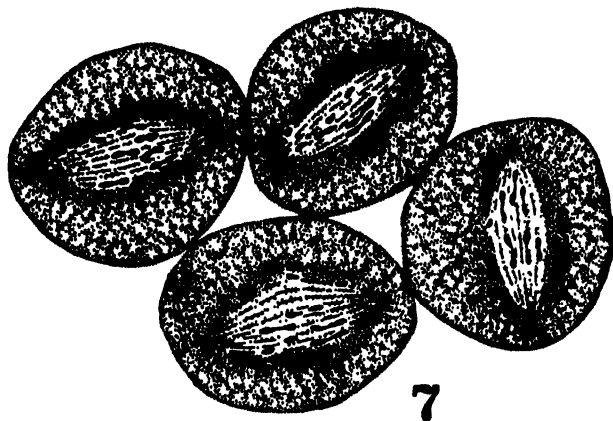
center of the body are found the much branched testicular organs. It is these which show most conveniently the maturation divisions in material fixed in the appropriate stages.

We may now turn our attention to the situation in tapeworms. Fig. 6 shows the general organization of



6

one of the segments in a tapeworm. Obviously, as in the fluke, the two kinds of reproductive organs are present in the same animal. Commonly the sperms develop more rapidly than the eggs, and in a fully matured segment of a tapeworm the spermaries are no longer present, the body being occupied mainly by developing eggs. Fig. 7 shows the meiotic conditions as illustrated by primary spermatocytes of *Moniezia expansa*, the common sheep tapeworm. The sperm mother cells in tapeworms, so far as they have been observed by the present writer, are extremely small and the reduction division is correspondingly difficult to discern. By using very thin sections, extremely brilliantly stained, it has been possible, however, to make out the essential features in the first division of the spermatocytes. Fig. 7 shows such a division extremely highly magnified by the use of a millimeter and a half Zeiss oil immersion, a reasonably powerful ocular, and a powerful ribbon filament light. It is clear that in all the four cells depicted in the drawing there is striking irregularity present. The chromosomes are in general bivalents, although some univalents seem



to be present. Instead of being gathered in the equator of the cell as is the normal situation for bivalents, and thus constituting the normal metaphase, they lie in various positions on the spindle and present in fact the atypical reduction division which is characteristic of many known hybrid plants. A similar condition has been described by the present author in the case of *Drosophila melanogaster*.⁵ The results in this case at first were strongly questioned by other observers, but now there is general agreement that the present author's results are correct, although there is still difference of opinion as to the conclusions which should be drawn from the extremely abnormal mitoses of this much investigated species.

The anaphase in the case of *Moniezia* is difficult to catch, but the same disorderly arrangement of chromosomes, which are now for the most part univalent, is present also in this stage. The secondary spermatocytes are so minute that it is extremely difficult even with the highest power to make out clearly the details of mitosis. It has not been thought necessary to figure them in the present connection, especially as the divisions of the secondary spermatocytes are in general much more regular than those of the primary ones.

⁵ "Evidence as to the Cause of So-called Mutations in *Drosophila*," *Genetica*, vii: 273-286.

It will be clear, if the above descriptions are correct, that in *Moniezia* extreme irregularities are present in the reduction division. An ever wider range of accumulated facts makes it more and more obvious that abnormalities in the reduction or meiotic or maturation divisions in plants and animals are of the greatest theoretical importance from the standpoint of the doctrine of descent. It is of interest to note in the case of the tapeworm under discussion that the reduction divisions present a close parallel to the reduction divisions in parthenogenetic flowering plants such as the dandelion, the hawkweed, the fleabane, the broomrape, etc., etc. It is practically universally conceded that the abnormalities in meiosis in hybrid flowering plants indicate hybrid origin for such forms. There can be little doubt that a similar conclusion should be drawn in the case of the paedogenetic parasites under discussion at the present time.

It will be of interest in the present connection to discuss the general relations of so-called diplogenesis. This phenomenon is presented by aphids, bees, wasps, flukes, tapeworms, etc., etc. It is sometimes not very aptly designated an alternation of generations. That term is best restricted to the conditions found in plants where there is a distinct alternation of generations, the two being, in general, cytologically distinct from one another. In the case of diplogenesis, however, although the method of reproduction varies from parthenogenetic to sexual between the phases, the cytological constitution in both types is the same. The diplogenesis of the Hymenoptera, aphids, etc., differs from that in the forms under discussion in the present article in one very important respect. The first-named forms are unisexual, whilst the parthenogenetic types under discussion in the present article are hermaphrodite. That constitutes an important difference and its theoretical significance has been recently pointed out by Wilson:

“*Special Peculiarities of the X-chromosome:* In the earlier stages of development, and in the division of the

somatic cells generally, the X-chromosome does not, so far as known, differ in behavior from the others, nor do the two sexes differ in this respect. In later stages, on the other hand, the X-chromosome in the male germ-line shows certain special peculiarities of behavior which sometimes appear in the spermatogonia and are almost always present in the spermatocytes. In the female line these differences do not exist, or are much less marked.”⁴

It is to be emphasized that Wilson has noted the distinction between the conduct of the chromosomes in the meiotic mitoses of parthenogenetic forms and the somatic mitoses. As far as the writer is aware he is the only zoologist who has clearly emphasized this important difference. The present writer on several occasions has pointed out that in known hybrids, however irregular the meiotic mitoses may be, the somatic divisions are in general quite normal. This parallelism suggests a fruitful comparison. It is very generally held on the plant side that parthenogenesis and indeed also apogamy are a sequel of hybridization. It would be strange if a different situation were presented by animals, especially as there is an enormous amount of supporting evidence for the hybrid hypothesis on the plant side. Wilson remarks, as indicated above, that the test of the sex chromosome theory of parthenogenesis, as held generally by zoologists, is presented by the meiotic mitoses in hermaphrodite animals. It will probably be clear to the reader who has perused the foregoing paragraphs that in the outstanding hermaphrodite types illustrated by the flukes and tapeworms the same lagging chromosomes are present as in unisexual forms. It clearly follows that such lagging chromosomes can not be properly regarded as sex chromosomes but that the most reasonable interpretation of them is that they constitute an abnormality following previous hybridization. This general statement seems to cover, however, only sex chromosomes of the

⁴“The Cell in Development and Heredity.” The Macmillan Company, New York. Third edition, with corrections. 1928.

univalent type. In those sex chromosomes which are represented by a diverse pair it is quite likely that there is some relation between the chromosomes and the function of sex. It will probably turn out to be true in the long run that all univalent sex chromosomes should not be designated as such, but should be considered as laggards indicating previous hybridization.

It would seem probable, then, that the cytological investigation of meiosis, in the two representative forms described in the present article, indicates that for animals as well as plants the explanation of parthenogenesis is previous hybridization. Although there may be a possible doubt in the case of unisexual animals, there can scarcely be any question where hermaphrodites such as *Fasciola* and *Moniezia* are concerned. When an extensive study of aphids has been completed it will be clear that current zoological hypothesis of parthenogenesis even in this group as dependent on sex chromosomes must be abandoned because of its unworkability.

The general investigation of meiosis in plants and animals seems to be destined to throw an extremely important light on the cause of evolutionary change. In the case of many animals and plants the maturation divisions are of what may be called a normal type, in which there are regular metaphases and anaphases. A marked departure from regularity is found in both hybrid and parthenogenetic types. It seems appropriate on the evidence here supplied to add to these diplogenetic and paedogenetic forms such as the tapeworms and flukes.

CHICK MORTALITY AND SEX-RATIO IN THE DOMESTIC FOWL

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It is a well-known fact that in man during childhood male mortality is higher than female mortality. This greater male mortality is responsible for a gradual decline of the sex-ratio from birth to puberty.

It appears that no observations have been made as yet concerning the relation between postnatal mortality and sex-ratio in the domestic fowl. Since such information aside from its immediate interest may be of theoretical value for the explanation of selective mortality in general, we have undertaken to analyze the mortality records for the first two months after hatching of the chicks, which at this station were hatched for experimental purposes during the years 1922 to 1930. Approximately 19,100 chicks were hatched during this nine-year period. About 9,500 chicks out of this total belonged to a large number of crosses which were made for the genetic analysis of various morphological characters, while the remaining 9,600 chicks were Single Comb White Leghorns. The Leghorn chicks belonged to inbreeding experiments carried out by Dr. L. C. Dunn and were partly derived from brother by sister or half-brother by sister matings and partly from crosses between unrelated individuals.

In tabulating the death records all cases of accidental death (due to crowding, predatory enemies, drowning, and so on) were omitted; these amounted to approximately 5 per cent. of the total mortality. There was also a small number of chicks which could not be sexed because they had lost their identification bands or were decomposed before they were found. Most of the chicks were hatched during March, April and May. There were some small hatches, however, which came off in January

TABLE I
MORTALITY RECORDS OF CROSS-BRED CHICKS 1922-1930
SEX-RATIO (PER CENT. OF MALES) AMONG CHICKS WHICH DIED DURING THE FIRST TWO MONTHS OF LIFE
9,500 chicks were hatched and the sex was recorded of 2,329 chicks dying from natural causes during the first two months of life

Year	First week			Second to fourth week			Fifth week to end of second month		
	Males	Females	Percentage males	Males	Females	Percentage males	Males	Females	Percentage males
1922	19	14	58	91	61	60	27	31	47
1923	46	36	56	212	198	52	40	31	56
1924	14	9	61	83	58	59	40	46	47
1925	12	4	75	40	46	47	35	30	54
1926	9	8	53	26	32	45	37	23	62
1927	28	20	58	51	39	57	51	40	56
1928	23	19	55	45	55	45	40	39	51
1929	29	36	45	72	57	56	28	28	50
1930	66	65	50	53	54	50	16	17	49
Totals	246	211	53.8	673	600	52.9	314	285	52.4

and February and in June and July. These were included, since several authors have shown that there is no significant seasonal variation in the sex-ratio of hatching chicks (Lambert and Knox, Horn, Lambert and Curtis). The mortality was tabulated separately for the first week after hatching, the second to fourth week, and from the beginning of the fifth week to the end of the second month of postnatal life. After the second month chick mortality usually was low and it was not expected that sufficient numbers could be accumulated from our records to detect significant differences between male and female mortality.

Tables I and II give the actual figures of males and females and the percentages of males which died in each year among the cross-bred and the White Leghorn chicks. The cross-breds with a total mortality of 2,329 chicks had a sex-ratio of dead chicks of 53.8, 52.9 and 52.4 per cent., respectively, during the first week, the second to fourth week, and the fifth week to the end of the second month intervals. The corresponding figures for the sex-ratio of dead chicks among 3,354 Leghorn chicks are 52.8, 51.6 and 54.6 per cent., respectively. For the combined material we have records of 5,683 dead chicks (from a total of 19,100 chicks hatched) with the following distribution:

	Males	Females	Percentage males	Difference from 50% P. E. of diff.
First week	539	473	53.3 $\pm$ 1.06	3.1
Second to fourth week	1676	1541	52.1 $\pm$ 0.59	3.7
Fifth week to end of second month	781	673	53.7 $\pm$ 0.88	4.2

The departures of these sex-ratios from equality exceed at least slightly three times their probable error. During the entire period of the first two months of life the male mortality amounted to 52.7 ± 0.45 per cent. This devia-

TABLE II
MORTALITY RECORDS OF LEGHORN CHICKS 1922-1929
SEX-RATIO (PER CENT. OF MALES) AMONG CHICKS WHICH DIED DURING THE FIRST TWO MONTHS OF LIFE
9,600 chicks were hatched and the sex was recorded of 3,354 chicks dying from natural causes during the first two months of life

Year	First week			Second to fourth week			Fifth week to end of second month		
	Males	Females	Percentage males	Males	Females	Percentage males	Males	Females	Percentage males
1922	14	6	70	45	32	58	54	34	61
1923	37	29	56	85	87	49	30	30	50
1924	65	43	60	453	396	53	134	110	55
1925	42	40	51	141	148	49	138	129	52
1926	16	18	47	43	51	46	29	11	73
1927	32	36	47	66	64	51	28	25	53
1928	11	14	44	87	76	53	39	33	54
1929	76	76	50	83	87	49	15	16	48
Totals	293	262	52.8	1003	941	51.6	467	388	54.6

tion from equality amounts to six times its probable error.

The significance of these deviations from chance mortality is considerably strengthened by the fact that there was a slight deficiency of male chicks already at hatching time. Dr. L. C. Dunn in an (unpublished) analysis of a part of the data used in this report found among a total of 5,421 Leghorn chicks 2,633 males and 2,788 females, corresponding to a sex-ratio of 48.57 ± 0.46 per cent. males. Among 2,638 cross-bred chicks he found 48.59 per cent. males. These figures are in close agreement with those reported by other investigators.¹

The sex records for the mortality of chicken embryos are conflicting. Some authors (Lambert and Knox, Horn) found a higher mortality of males, while others (Jull, Lambert and Curtis) observed a greater female mortality. Probably in no case a sufficient number of embryos has been observed to detect significant deviations from an equal mortality of the two sexes. If in the fowl there is equality of the sexes at fertilization (primary sex-ratio), as all authors seem to assume, then the significant deficiency of males among almost 68,000 chicks observed at hatching time would suggest that there is a slight majority of males among the embryos which die. At any rate, it seems safe to say that if any differential mortality occurs during embryonic development of chickens, it is the male sex which suffers more; if this is so, the same conditions which from our records appear to prevail in postnatal life, would already exist before hatching.

¹ The combined figures of all available observations (Darwin, Field, Pearl, Crew and Huxley, Jull, Mussehl, Lambert and Knox, Horn, Lambert and Curtis, Dunn, Callenbach, Christie and Wriedt, and Jull) of the sex of chicks at hatching amount to 67,993 chicks with 33,162 males, corresponding to 48.77 ± 0.13 per cent. males. This deficiency of males exceeds nine times its probable error, and it can not be doubted, therefore, that in general there is already a deficiency of male chicks at hatching time.

It appears that no evidence is available at present which can be used for the explanation of the greater male mortality among human infants. Lenz, Huxley and Schirmer have put forward the hypothesis that recessive sex-linked factors with a slightly deleterious effect upon the viability account for the greater male mortality. Huxley says: "Since the male mammal is heterogametic, any recessive factor borne in the X-chromosome will take effect in all males carrying them, whereas in females both sex-chromosomes must carry the factor before the corresponding characters appear." Huxley believes that circumstantial evidence for the correctness of his explanation is to be found in the fact that adverse conditions seem to intensify, favorable ones to neutralize the differential male mortality during pregnancy (Parkes, Punnett). Furthermore, he thinks that this explanation "also provides a basis for the fact that male secondary sex-ratio is higher in the offspring of young than of old mothers and higher in first births, decreasing at each subsequent pregnancy." The lower male secondary sex-ratio of illegitimate children and the higher one of the Jewish population, Huxley likewise explains with this hypothesis, assuming that differences in prenatal care will aggravate or counteract to a certain extent the harmful effects of recessive sex-linked factors. The higher percentage of males found by Little (and before him by Pearl and Pearl) among children of wide racial crosses as compared with the sex-ratio of relatively pure stock is explained by Huxley as being due to heterosis, "enabling the males to resist the deleterious effect of harmful sex-limited factors."

If harmful sex-linked factors are assumed to be the cause of higher male mortality in human infants (and embryos), then we should expect to find the reverse situation, higher female mortality, in animals like chickens in which the females are the heterogametic sex. Actually, however, we saw that in chickens as in man the post-

natal mortality is higher in males than in females. If there is in chickens any differential mortality during embryonic development, as is suggested by the significant deficiency of males at hatching, then again it appears that the situation is the same as in man. In view of our evidence from chickens the general explanatory value of the hypothesis of Lenz, Huxley and Schirmer becomes rather doubtful, although it is quite possible that sex-linked factors play a minor rôle as a cause of differential mortality. We must look for an explanation which can be applied equally well to man and fowls.

For most, if not all, classes of higher animals it appears to be characteristic that the males have a higher basal metabolism than the females. The work of Riddle and others makes it probable that this metabolic difference in one way or another already begins during embryonic development. The life span of any mechanical engine under otherwise constant conditions depends upon the speed at which it is run. Speed that falls above or below the optimum means greater wear.² The assumption that generally the basal metabolism of the female sex approaches optimal conditions more closely than that of the male would furnish an explanation for the greater infant mortality of organisms as far apart as man and domestic fowls. In a physico-chemical system the life span of a mechanism under otherwise constant conditions varies with the rate at which reactions take place. Seen from this view-point, the male again would be at a disadvantage. The acceleration of chemical reactions corresponding to the higher metabolic rate must mean greater wear on the physical parts of the organic machine. It appears also from recent work with pigeons (Riddle, Christman and Benedict) that the basal metabolism of males is more easily and to a greater extent upset by unfavorable conditions than is that of

² There are probably engines in which the optimum is at or near the minimum speed. This, however, is taken as a special case of the general rule.

females. This finding points to the conclusion that in the male organism processes take place with a lesser degree of stability which again would involve a greater amount of wear through necessary readjustments. There is considerable evidence in favor of such an explanation of the differential mortality of males and females, most of which may be found in Joyet-Lavergne's recent book. Working with *Daphnia magna* MacArthur and Baillie found that the males have a higher metabolic rate than the females. They found, furthermore, that the males of this species have a more rapid heart beat than the females. On the basis of this and other evidence MacArthur and Baillie suggest that the shorter life span of the males of *Daphnia magna* is to be explained as a consequence of the existing metabolic differences.

In addition to this instance in which a direct association could be demonstrated to exist between the metabolic rate and the speed of functioning of an organ there is ample evidence for the conclusion that the higher metabolic rate of males is not compensated by a different organization of the organism, but is actually brought about by a more rapid or more continuous functioning of the organs and cells of the male body. Most of the work, for instance, that has been done in connection with Manoiloff's reaction points to this conclusion.

It appears that there are no observations on record which are inconsistent with the assumption that the higher metabolic rate of males is produced by a more rapid or more continuous performance of organs and cells of the male organism, and if the latter conclusion is valid, our comparison between the life span of organisms and of mechanical engines appears reasonable.

The observations which Lenz, Huxley and Schirmer adduce to strengthen their hypothesis would fit equally well into an explanation on the basis of differences in metabolism—as, in fact, they would fit into almost any other physiological explanation. Until new evidence is

forthcoming, these sexual differences in the rate and stability of metabolism are offered as a working hypothesis for the understanding of differential mortality in the two sexes.

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LINKAGE IN SIZE INHERITANCE

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IN reports of a previous investigation on size inheritance and growth in a mouse species cross, the author (1930, 1931) noted indications of linkage between general size and color characters. Since such linkage had never been demonstrated in mammals it was considered advisable to continue work along this line in an effort either to confirm or to disprove its presence.

The animals used in the present investigation were of the same stocks as those previously employed: a small Chinese species, *Mus bactrianus*, and a large inbred race of *Mus musculus*. The former when adult weigh but little more than half as much as the latter, while all other quantitative characters studied likewise have lower values. Tables showing mean values for quantitative indices of the two species were given in one of the papers cited (1931). The two forms still retain the same relationships, although for some reason not clearly understood the absolute values of both are now lower.

In addition to size differences the smaller species possesses the dominant color genes for white bellied agouti (A^*), black (B) and intensity (D); while the larger has the recessive allelomorphs, non-agouti (a), brown (b) and dilution (d). Herein lies an especial advantage for the detection of linkage. Since recessive genes are generally more deleterious than dominant, any tendency of our back-cross mice to vary in the direction of their respective parental types can not be attributed to the beneficial effects of dominant genes.

Since the purpose of the renewed investigation was the demonstration of linkage—if such existed—between quantitative and qualitative characters, the back-cross generation was used almost exclusively, with in addi-

tion, however, a small F_2 generation. The back-cross was made in only one way. *Musculus* females were mated to F_1 males, all of which in turn were the product of *musculus* mothers and *bactrianus* fathers. All back-cross and F_2 animals were born between July 10 and December 29, 1930. Diet and care were kept uniform throughout the experiment. The mice were killed between the 181st and the 184th day and the external measurements taken immediately after death. The leg bones and the skulls were cleaned of flesh by boiling in a solution of ammonium, phenol and water. After the cleaned bones had dried at least two days in the open air the skeletal measurements with the exception of skull width (interorbital width) were taken with a Starrett bench micrometer to the nearest .01 mm. For skull width vernier calipers were used, the value being recorded to the nearest .1 mm. The author personally prepared all the bones and made all the measurements, thus reducing the personal error incident to more than one observer and recorder.

The quantitative characters reported on in this paper comprise the following: weight on the first, eleventh, thirty-first, sixty-first, ninety-first, one hundred twenty-first, one hundred fifty-first and one hundred eighty-first day; body length, tail length, skull length, skull width, humerus length, femur length, tibia length and cranial capacity. These measurements are described in an earlier paper by the author (1931a), so detailed descriptions are unnecessary here.

As before mentioned, the existence of linkage of size—relating to the organism as a whole and not to a particular part—with color characters has never been conclusively proved in mammals. Castle (1929) could find no evidence for it in rabbits, nor could Livesay (1930) in rats. Among plants, Lindstrom (1926), for example, found fruit color linked with fruit size in tomatoes. The same author (1929) showed that the number of rows in the maize ear was associated in inheritance with several

simple mendelizing characters such as cob-color and endosperm color. These examples, however, are perhaps scarcely comparable with the usual situation in mammals, since they refer to a particular part of the organism rather than to the general size, of which weight, body length and bone lengths are more or less satisfactory manifestations.

If, in our investigation, the "tagged" *musculus* chromosomes carrying the recessive genes for color also possess genes influencing size then the back-cross mice with the recessive factors will tend to be larger than those with the dominant allelomorphs. If quantitative characters are not inherited through chromosomal genes or if no such genes are present on the three chromosomes investigated the recessive members of the factor pairs will exhibit no tendency to exceed the dominant. Thus in determining the presence or absence of linkage, all agouti back-cross mice were compared with all non-agouti, in regard to each of the quantitative characters. Similarly, blacks were compared with browns and intense animals with dilute. The sexes, of course, were considered separately.

Tables I and II present these mean values as well as the means for the total population of that generation. None of the mice were used for breeding, so all females included were virgins.

Table III presents a summary of the significant differences in adult quantitative characters between the recessive and dominant members of the factor pairs. A difference as great as or greater than four times its probable error is considered significant.

In the matter of weight, brown mice of both sexes are significantly heavier than blacks at the age of 181 days. A perusal of Table I, however, shows that this condition does not prevail at all ages, since in early life the situation is reversed, perhaps because of the initial effects of the dominant gene. In neither of the other factor pairs is there a significant difference in adult weight.

TABLE I
COMPARATIVE WEIGHTS OF DIFFERENT CLASSES OF BACK-CROSS MICE

Class	1st day δ δ			Weight (gms) δ δ			11th day δ δ			Weight (gms) δ δ			31st day δ δ			Weight (gms) δ δ			61st day δ δ			Weight (gms) δ δ		
	No.	Mean		No.	Mean		No.	Mean		No.	Mean		No.	Mean		No.	Mean		No.	Mean		No.	Mean	
gouti	85	1.36 $\pm$.014		70	1.29 $\pm$.015		85	5.12 $\pm$.071		70	4.89 $\pm$.075		85	9.19 $\pm$.150		70	8.44 $\pm$.150		85	15.2 $\pm$.16		70	13.3 $\pm$.15	
on-agouti ..	67	1.30 $\pm$.014		70	1.23 $\pm$.015		68	4.96 $\pm$.056		70	4.76 $\pm$.059		68	8.57 $\pm$.148		70	8.26 $\pm$.129		68	14.9 $\pm$.18		70	13.3 $\pm$.14	
black	81	1.37 $\pm$.014		62	1.28 $\pm$.016		81	5.24 $\pm$.070		62	4.90 $\pm$.073		81	8.93 $\pm$.159		62	8.16 $\pm$.144		81	14.8 $\pm$.17		62	13.1 $\pm$.15	
rown	71	1.30 $\pm$.014		78	1.24 $\pm$.015		72	4.84 $\pm$.057		78	4.76 $\pm$.064		72	8.81 $\pm$.134		78	8.49 $\pm$.135		72	15.4 $\pm$.16		78	13.5 $\pm$.13	
itense	81	1.35 $\pm$.014		67	1.27 $\pm$.015		82	5.08 $\pm$.059		67	4.82 $\pm$.067		82	9.05 $\pm$.147		67	8.15 $\pm$.143		82	15.1 $\pm$.16		67	13.0 $\pm$.14	
ilute	71	1.31 $\pm$.014		73	1.25 $\pm$.016		71	5.02 $\pm$.075		73	4.83 $\pm$.069		71	8.76 $\pm$.154		73	8.52 $\pm$.136		71	15.0 $\pm$.18		73	13.6 $\pm$.15	
Total	152	1.33 $\pm$.009		140	1.26 $\pm$.011		153	5.05 $\pm$.047		140	4.82 $\pm$.048		153	8.91 $\pm$.113		140	8.34 $\pm$.099		153	15.1 $\pm$.12		140	13.3 $\pm$.10	

Class	91st day δ δ			Weight (gms) δ δ			121st day δ δ			Weight (gms) δ δ			151st day δ δ			Weight (gms) δ δ			181st day δ δ			Weight (gms) δ δ		
	No.	Mean		No.	Mean		No.	Mean		No.	Mean		No.	Mean		No.	Mean		No.	Mean		No.	Mean	
gouti	85	16.8 $\pm$.16		70	15.0 $\pm$.17		85	18.6 $\pm$.17		70	16.8 $\pm$.18		85	20.1 $\pm$.16		70	18.1 $\pm$.24		85	21.4 $\pm$.20		70	19.2 $\pm$.27	
on-agouti ..	68	17.2 $\pm$.21		70	15.0 $\pm$.15		68	18.9 $\pm$.25		70	16.9 $\pm$.17		68	20.6 $\pm$.28		70	18.5 $\pm$.22		68	22.0 $\pm$.30		70	19.7 $\pm$.28	
ack	81	16.4 $\pm$.17		62	14.4 $\pm$.18		81	18.2 $\pm$.18		62	16.0 $\pm$.17		81	19.6 $\pm$.20		62	17.3 $\pm$.19		81	21.0 $\pm$.22		62	18.3 $\pm$.24	
own	72	17.6 $\pm$.18		78	15.6 $\pm$.14		72	19.4 $\pm$.23		78	17.5 $\pm$.16		72	21.0 $\pm$.22		78	19.1 $\pm$.24		72	22.8 $\pm$.26		78	20.4 $\pm$.25	
tense	82	17.1 $\pm$.17		67	14.7 $\pm$.16		82	18.9 $\pm$.22		67	16.6 $\pm$.17		82	20.4 $\pm$.21		67	18.0 $\pm$.25		82	21.9 $\pm$.26		67	19.3 $\pm$.27	
lute	71	17.0 $\pm$.18		73	15.4 $\pm$.16		71	18.6 $\pm$.19		73	17.0 $\pm$.18		71	20.2 $\pm$.22		73	18.6 $\pm$.22		71	21.5 $\pm$.23		73	19.6 $\pm$.26	
Total	153	17.0 $\pm$.13		140	15.1 $\pm$.12		153	18.8 $\pm$.15		140	16.8 $\pm$.12		153	20.3 $\pm$.15		140	18.3 $\pm$.16		153	21.7 $\pm$.17		140	19.5 $\pm$.19	

TABLE II
COMPARATIVE MEASUREMENTS OF DIFFERENT CLASSES OF BACK-CROSS MIXTURES

Class	Skull length (mm)			Skull width (mm)			Humerus length (mm)			Femur length (mm)		
	No.	Mean	Sex	No.	Mean	Sex	No.	Mean	Sex	No.	Mean	Sex
Agouti	85	21.06 ± .036	♀	85	3.80 ± .008	♀	85	11.02 ± .028	♀	85	14.28 ± .042	♀
Non-agouti	69	20.95 ± .032	♂	70	3.79 ± .010	♂	67	10.88 ± .028	♂	66	14.16 ± .035	♂
Black	81	20.98 ± .037	♀	81	3.78 ± .008	♀	80	10.83 ± .028	♀	80	14.08 ± .038	♀
Brown	72	21.05 ± .033	♂	72	3.80 ± .010	♂	72	11.10 ± .026	♂	71	14.39 ± .027	♂
Intense	82	20.96 ± .036	♀	82	3.78 ± .008	♀	81	10.91 ± .030	♀	82	14.16 ± .038	♀
Blute	71	21.07 ± .034	♂	73	3.82 ± .009	♂	73	11.02 ± .028	♂	69	14.30 ± .040	♂
Total	153	21.01 ± .025		153	3.79 ± .007		152	10.96 ± .021		151	14.22 ± .028	

Class	Tibia length (mm)			Body length (mm)			Tail length (mm)			Cranial capacity (gms of Hg.)		
	No.	Mean	Sex	No.	Mean	Sex	No.	Mean	Sex	No.	Mean	Sex
Agouti	85	16.27 ± .043	♀	85	89.2 ± .24	♀	83	86.3 ± .35	♀	85	5.15 ± .019	♀
Non-agouti	67	16.08 ± .034	♂	69	15.83 ± .042	♂	66	86.9 ± .42	♂	68	5.09 ± .021	♂
Black	80	16.08 ± .041	♀	81	88.4 ± .25	♀	80	86.8 ± .37	♀	81	5.15 ± .021	♀
Brown	72	16.30 ± .036	♂	72	89.9 ± .21	♂	69	86.3 ± .40	♂	72	5.09 ± .019	♂
Intense	81	16.09 ± .039	♀	82	88.5 ± .25	♀	78	85.0 ± .33	♀	82	5.11 ± .020	♀
Blute	71	16.30 ± .038	♂	73	89.9 ± .22	♂	71	88.2 ± .40	♂	71	5.13 ± .020	♂
Total	152	16.18 ± .028		153	89.1 ± .17		149	86.6 ± .27		153	5.12 ± .014	

TABLE III
COMPARISON OF RECESSIVE AND DOMINANT MEMBERS OF FACTOR PAIRS. ADULT QUANTITATIVE CHARACTERS OF BACK-CROSS MICE

Character	Recessive	Mean	Dominant	Mean	Difference	Difference Probable error
Weight, 181st day	Brown ♂ ♂ Brown ♀ ♀	22.8 ± .26 gms 20.4 ± .25 gms	Black ♂ ♂ Black ♀ ♀	21.0 ± .22 gms 18.3 ± .24 gms	1.8 ± .34 gms 2.1 ± .35 gms	5.3 6.0
Skull length	No significant differences					
Skull width	No significant differences					
Humerus length	Brown ♂ ♂ Brown ♀ ♀	11.10 ± .026 mm 10.74 ± .027 mm	Black ♂ ♂ Black ♀ ♀	10.83 ± .028 mm 10.52 ± .029 mm	.27 ± .038 mm .22 ± .040 mm	7.1 5.5
Femur length	Brown ♂ ♂ Brown ♀ ♀	14.39 ± .037 mm 14.24 ± .037 mm	Black ♂ ♂ Black ♀ ♀	14.08 ± .038 mm 13.95 ± .038 mm	.31 ± .053 mm .29 ± .053 mm	5.8 5.5
Tibia length	Brown ♂ ♂ Brown ♀ ♀	16.30 ± .036 mm 16.02 ± .036 mm	Black ♂ ♂ Black ♀ ♀	16.08 ± .041 mm 15.76 ± .042 mm	.22 ± .055 mm .26 ± .055 mm	4.0 4.7
Body length	Brown ♂ ♂ Brown ♀ ♀ Dilute ♂ ♂ Dilute ♀ ♀	89.9 ± .21 mm 89.0 ± .23 mm 89.9 ± .22 mm 89.0 ± .26 mm	Black ♂ ♂ Black ♀ ♀ Intense ♂ ♂ Intense ♀ ♀	88.4 ± .25 mm 87.5 ± .27 mm 88.5 ± .25 mm 87.6 ± .23 mm	1.5 ± .33 mm 1.5 ± .35 mm 1.4 ± .33 mm 1.4 ± .35 mm	4.5 4.2 4.2 4.0
Tail length	Dilute ♂ ♂ Dilute ♀ ♀	88.2 ± .40 mm 86.0 ± .45 mm	Intense ♂ ♂ Intense ♀ ♀	85.0 ± .33 mm 84.3 ± .37 mm	3.2 ± .52 mm 1.7 ± .58 mm	6.2 2.9
Cranial capacity	No significant differences					

Skull length and skull width show no significant differences.

In humerus length, on the other hand, browns unquestionably surpass blacks. They likewise show significantly greater femur and tibia lengths than do animals with the dominant allelomorph.

Browns significantly exceed blacks in body length. Dilute animals, it will be observed, similarly differ from intense in body length and probably also in tail length.

Cranial capacities exhibit only unimportant differences.

The relations of black to brown back-cross animals in regard to humerus length, body length and adult weight are depicted graphically by frequency polygons in Figs. 1 to 4.

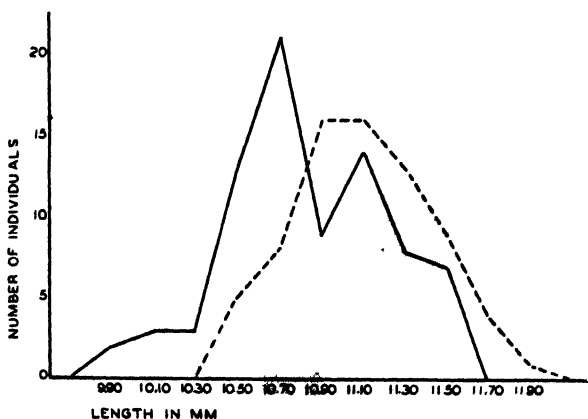


FIG. 1. Humerus length. Back-cross males. Solid line, blacks; broken line, browns.

From the data in Tables II and III, it is evident that several size characters, namely, humerus, femur and tibia lengths, adult weight and body length, are influenced by factors linked with the gene for brown. It also appears that other factors influencing body length and probably tail length are found on the chromosome with dilution. Apparently no special factors affecting skull length, width or cranial capacity are located on any of the "tagged" *musculus* chromosomes.

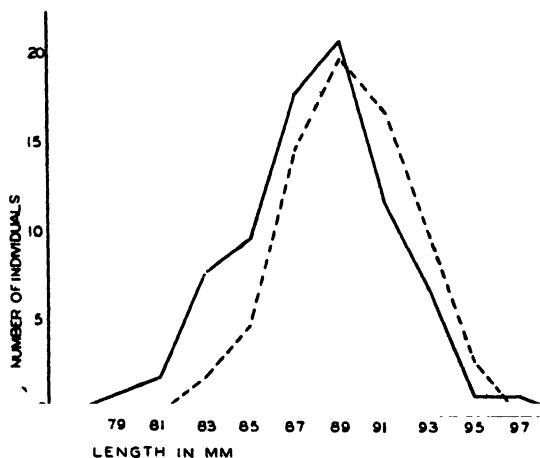


FIG. 2. Body length. Back-cross males. Solid line, blacks; broken line, browns.

Since both femur length and tibia length are markedly influenced by factors, either common or specific, linked with brown, while skull length apparently is not so influenced, the two leg bone lengths should prove to be more closely correlated than is either with skull length. The coefficients of correlation given on page 510 bear out this assumption.

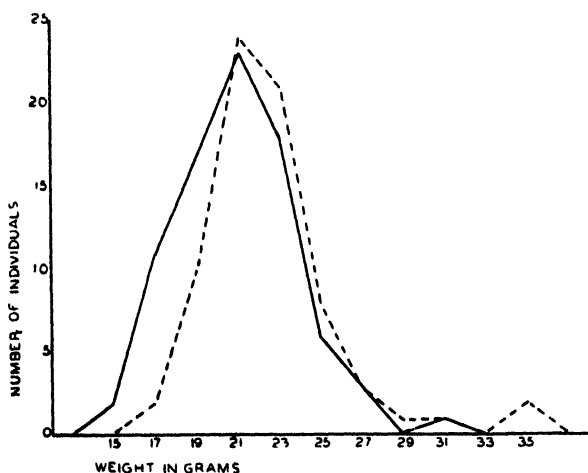


FIG. 3. 181st day weight. Back-cross males. Solid line, blacks; broken line, browns.

	Femur length— Tibia length	Femur length— Skull length	Difference	Difference P. E.
Males	$r = +.839 \pm .016$	$r = +.689 \pm .029$	$.150 \pm .033$	4.5
Females ...	$r = +.841 \pm .017$	$r = +.678 \pm .031$	$.163 \pm .035$	4.7

A peculiar situation is revealed in the comparison of agouti and non-agouti: Mice with the former character, which is a dominant and comes into the cross with a chromosome from the smaller species, tend to exceed non-agouti in size. In no case, however, was this difference great enough to reach significance under our criterion. The agouti gene may have some innate beneficial physiologic effect or perhaps it may be linked with other

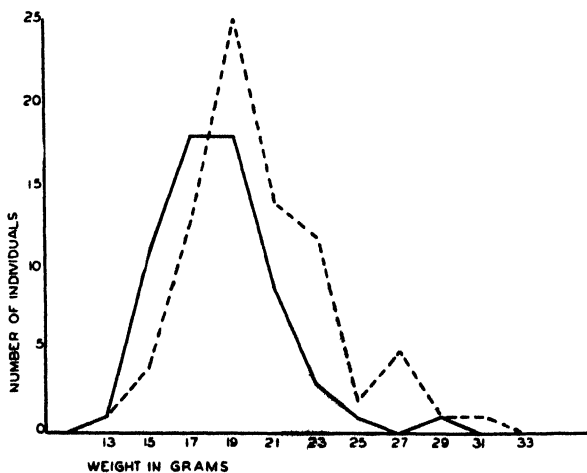


FIG. 4. 181st day weight. Back-cross females. Solid line, blacks; broken line, browns.

advantageous genes. This may give a clue as to the reason for the prevalence of the agouti pattern among wild rodents.

A very small F_2 generation consisting of only 24 animals presents no evidence antagonistic to the findings in the back-cross generation, although of course the numbers are too small to be of any significance. For every adult size character, the average value of the three brown

males and of the three brown females exceeded that of the eight black males and of the ten black females, respectively.

SUMMARY

An analysis of the data on the back-cross generation of a mouse interspecific cross between large *Mus musculus*, with three recessive color characters, and small *Mus bactrianus*, with the three dominant allelomorphs, has indubitably shown an association in heredity between factors productive of a large size in several quantitative characters and a recessive qualitative character, brown coat color. Thus, the fact that size in mice is influenced by chromosomal genes can scarcely be questioned any longer. To a lesser extent factors linked with dilution also affect size in certain characters.

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THE PROBLEM OF UNFRUITFULNESS IN THE CULTIVATED APPLE¹

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RECENTLY ('29), Wellington, Stout, et al, made this statement concerning the cultivated apple: "Fruit production is complex and dependent upon many factors. . . . The main factors affecting fruit setting may be roughly classified into five categories, namely, meteorological, pathological, nutritional, sexual and agencies effecting pollination." However important the other factors may be, we are concerned here chiefly with the sexual phenomena.

Although Kölreuter, in 1764 (East '29), appears to have been the discoverer of self-sterility in plants, it was not until 1898 that self-sterility (self-unfruitfulness)² was discovered in the apple. Waite ('98) of the U. S. Department of Agriculture published an account of his experiments upon barren orchards in Virginia. He concluded that certain varieties of pomaceous fruits were unable to set with their own pollen but that cross-pollination was effective in most cases.

Barring, then, unfruitfulness, due to one or more of the causes other than sexual, the problem that Waite pre-

¹ Acknowledgment is due Dr. O. E. White, under whose direction this work was done while the writer was a Blandy Fellow at The Blandy Experimental Farm and a student at the Miller School of Biology, University of Virginia.

² The terms self-fruitful and self-unfruitful have recently taken the place of self-fertile and self-sterile, respectively, in correct horticultural usage. "Chittenden ('14) recalls that self-fertile in its restricted sense implies that viable seed is produced, while in a wider sense, and one that concerns the fruit grower, it means that the pericarp or fleshy envelope of the fruit is formed. There may or may not be any seeds enclosed within it. He proposes, therefore, the term of self-fruitful instead of self-fertile for this latter class, restricting the term self-fertile only to the cross where seed is produced. With this terminology, a fruit tree may at the same time be self-fruitful and self-sterile, or self-fruitful and self-fertile." Quotation from Einset ('30).

sented to horticulturists and later to geneticists was to find which apple varieties were self-fruitful and in which varieties cross-pollination was necessary.

Since then, the problem has been worked at diligently, and although Waite is undoubtedly correct in several cases, a solution to the problem does not seem much nearer now than it did then. The statement made by Fletcher (1900) seems to be as appropriate now as it was thirty years ago, "Self-sterility (self-unfruitfulness) is not a constant character with any variety. It is influenced by conditions under which the tree is grown. . . . No one can separate varieties into two definite classes which are self-sterile. The problem of self-sterility is as much a study of conditions as of varieties. We can set no limits; we can only indicate tendencies."³

East ('29) sums up the present position thus: "The situation appears to be that numerous varieties of apples and pears are self-sterile (self-unfruitful)⁴ under certain climatic conditions, but are self-fertile under other conditions. . . . The most disturbing phenomenon from the horticultural point of view, however, is the fact that self-sterile varieties ordinarily needing to be crossed with compatible sorts, if merchantable crops are to be produced, sometimes will produce excellent crops when the trees are grown in isolated blocks."

The following table made by Murneek et al ('30) which is an excellent summary of the literature for some of the most important varieties, shows clearly the inconsistency and confusion arising from the results.

The above table is made from the separate works of twenty-five investigators and includes eighteen varieties. Of the 115 accounts on these varieties, only 12 show the necessary 5 per cent. set for a commercial yield. Contrast the accounts of Vincent of Idaho on Yellow Transparent (33.6 per cent.) self-fruitful, with Auchter's of Maryland and Morris' of Washington reports of 0.0 per

³ From Murneek, et al ('30).

⁴ Brackets are mine.

TABLE I
RANGE OF SELF-FRUITFULNESS OF SOME IMPORTANT VARIETIES OF APPLES

Variety	Investigator	Locality	Number of blossoms considered	Percentage of blossoms set
"	Lewis and Vincent ('09)	Oregon	†	0.0
"	Auchter ('21)	Maryland	1,909	0.0
"	"	Maryland	543 (s) ⁵	0.0
"	Auchter and Schrader ('25)	Maryland	500 (s)	0.0
"	Knowlton ('27)	West Virginia	230 (s)	0.0
Arkansas, black	Lewis and Vincent	Oregon	†	0.0
"	Vincent ('20)	Idaho	448	0.0
"	Auchter	Maryland	2,620	0.0
"	Auchter	Maryland	228 (s)	0.0
"	Lace and Morris ('28)	Washington	118 (s)	0.0
Ben Davis	Lewis and Vincent	Oregon	†	3.0
"	Wicks ('18)	Arkansas	472 (s)	2.3
"	Gowen ('20)	Maine	339	0.0
"	Vincent	Idaho	708	1.2
"	Morris ('21)	Washington	509	0.2
"	Sax ('22)	Maine	1,695	0.4
Delicious	Vincent	Idaho	231	0.0
"	Dorsey ('21)	Minnesota	73 (s)	0.7
"	Crandall ('22)	Illinois	†	0.0
"	Morris	Washington	530	0.0
"	Whitehouse and Auchter ('26)	Maryland	687 (s)	0.0
"	Howlett ('27)	Ohio	200 (s)	0.5
"	Overholser ('27)	California	426 (s)	0.0
"	Lace and Morris	Washington	263 (s)	0.0
"	Marshall, et al ('29)	Michigan	564 (s)	0.0
"	Lewis and Vincent	Oregon	†	5.0
Puchess	Chittenden ('14)	England	348	0.3

⁵ Covered and selfed; other figures for covered only.

TABLE I—(Continued)

Variety	Investigator	Locality	Number of blossoms considered	Percentage of blossoms set
Duchess	Logsdail ('17)	Ontario	479	0.0
"	Vincent	Idaho	381	19.0
"	Dorsey	Minnesota	271	0.0
"	Morris	Washington	253	11.5
"	Crandall	Illinois	† (s)	0.0
"	Macoun ('22)	Canada	530	11.5
"	Florin ('27)	Sweden	513 (s)	0.0
"	Marshall, et al	Michigan	2,162 (s)	0.0
"	Powell ('02)	Delaware	408	5.9
Early Harvest	Gowen	Maine	13	0.0
"	Vincent	Idaho	152	1.3
"	Crandall	Illinois	†	0.0
"	Lewis and Vincent	Oregon	†	0.0
Gano	Logsdail	Ontario	318	0.0
"	Vincent	Idaho	668	3.6
"	Auchter	Maryland	1,173	0.1
"	Howlett	Maryland	607 (s)	0.5
"	Knowlton	Ohio	276 (s)	0.0
Golden Delicious	Powell	West Virginia	213 (s)	1.0
"	Lewis and Vincent	Delaware	135	0.0
Grimes	Sutton ('18)	Oregon	†	14.0
"	Wicks	England	36 (s)	0.0
"	Vincent	Arkansas	442 (s)	8.0
"	Auchter	Idaho	10,765	2.2
"	Morris	Maryland	661	1.7
"	Macoun	Maryland	662 (s)	0.1
"	Keil ('23)	Washington	2,484	1.5
"		Canada	24	0.0
"		Ohio	720 (s)	0.0

TABLE I—(Continued)

Variety	Investigator	Locality	Number of blossoms considered	Percentage of blossoms set
Grimes	Howlett	Ohio	148 (s)	0.7
"	Marshall, et al.	Michigan	670 (s)	0.3
Jonathan	Lewis and Vincent	Oregon	?	0.0
"	Wicks	Arkansas	452 (s)	3.8
"	Vincent	Idaho	19,081	2.9
"	Dorsey	Minnesota	188 (s)	2.1
"	Morris	Washington	504	0.0
"	Howlett	Ohio	174 (s)	0.0
"	Overholser	California	600 (s)	0.4
"	Luce and Morris	Washington	282 (s)	3.5
"	Marshall, et al.	Michigan	535 (s)	0.7
King David	Vincent	Idaho	605	0.5
"	Dorsey	Minnesota	195 (s)	0.0
"	Lewis and Vincent	Oregon	?	0.0
Ralls	Keil	Ohio	720 (s)	0.0
"	Lewis and Vincent	Oregon	?	0.0
Rome	Alderman ('17)	West Virginia	16,826 (s)	1.0
"	Logsdail	Ontario	166	0.0
"	Vincent	Idaho	10,326	4.5
"	Keil	Ohio	720 (s)	0.9
"	Howlett	Ohio	80 (s)	2.5
Stayman	Luce and Morris	Washington	110 (s)	10.0
"	Powell	Delaware	106	?
"	Auchter	Maryland	845	0.0
"	Auchter	Maryland	560 (s)	0.0
"	Crandall	Illinois	?	0.0
"	Howlett	Ohio	70 (s)	0.0
"	Knowlton	West Virginia	1,795	1.6
"	Luce and Morris	Washington	216 (s)	0.0
Wealthy	Waugh ('98)	Vermont	28	0.0

TABLE I—(Continued)

Variety	Investigator	Locality	Number of blossoms considered	Percentage of blossoms set
Wealthy	Lewis and Vincent	Oregon	†	0.0
"	Chittenden	England	30	0.0
"	Logsdail	Ontario	172	2.0
"	Vincent	Idaho	351	3.7
"	Auchter	Maryland	1,059	4.5
"	Morris	Maryland	799 (s)	1.9
"	Macoun	Washington	647	0.5
"	Keil	Canada	125	6.4
"	Howlett	Ohio	720 (s)	0.0
"	Marshall, et al.	Ohio	84 (s)	0.0
"	Powell	Michigan	658 (s)	0.8
Winesap	Lewis and Vincent	Delaware	300	0.0
"	Wicks	Oregon	†	0.0
"	Vincent	Arkansas	500	0.4
"	Morris	Idaho	365	0.0
"	Crandall	Washington	1,096	1.6
"	Luce and Morris	Illinois	†	0.0
Yellow Transparent	Powell	Washington	910 (s)	0.0
"	Lewis and Vincent	Delaware	363	5.5
"	Logsdail	Oregon	†	8.0
"	Vincent	Ontario	605	0.9
"	Auchter	Idaho	107	33.6
"	Morris	Maryland	514	2.7
"	Florn	Maryland	42 (s)	0.0
"	Powell	Washington	510	0.0
"	Lewis and Vincent	Sweden	607	1.2
"	Alderman	Delaware	134	0.0
York		Oregon	†	0.0
"		West Virginia	21,742 (s)	0.6

cent. The difference in locality can hardly be expected to account for so much variation in results.

Three definite conclusions can be drawn from this table:

(1) Self-pollinated apple trees can not as a rule be depended upon to produce a satisfactory crop.

(2) Some varieties are more self-fruitful than others.

(3) The inconsistency of results indicate that there is some discrepancy in this method of attack and that all the factors that enter into the set of the apple are not accounted for.

Hoping to throw some further light upon this complex subject, the writer during the years 1928-1929, at The Blandy Experimental Farm of the University of Virginia, attempted to find the extent of self-unfruitfulness existing among the different apple varieties at hand.

Following the method in vogue, unopened flowers from the varieties to be used as pollen parents were secured and left covered in a dry place until the anthers dehisced. This pollen was applied with a camel's hair brush to the unopened emasculated flowers of the variety to be used as the female parent. These pollinated flowers were not covered since several experiments both by myself and others (Sax '22) have proved that bees do not visit flowers so treated. Wind pollination is a negligible factor in the apple.

The composite results on self-compatibility for the two years are as follows:

TABLE II

Of 44 self-pollinated	Grimes Golden	flowers 2 set or 4.5 per cent.
" 170 " "	York Imperial	" 5 set or 2.9 per cent.
" 79 " "	Stark's Delicious	" 1 set or 1.2 per cent.
" 41 " "	Stayman's Winesap	" 0 set or 0.0 per cent.
" 39 " "	Winesap	" 0 set or 0.0 per cent.
" 183 " "	Baldwin	" 1 set or 0.5 per cent.
" 55 " "	Ben Davis	" 0 set or 0.0 per cent.

This table except for the results on Baldwin merely increases the range of Table I.

Howlett ('27) of Ohio eliminated experimental error almost entirely in the case of Baldwin, when he enclosed one whole tree under a muslin frame with a hive of bees. From these self-pollinated flowers he received a 5 per cent. set whereas an open pollinated tree nearby gave a 25 per cent. set.

The literature on cross-compatibility is even more confusing and inconsistent than that on self-compatibility. A particular variety may be reported as an effective pollenizer for another particular variety or group of varieties by one investigator and the reverse is found by another worker. However they all agree that the pollen of a variety is generally more effective upon the flowers of some other variety than upon its own.

In Table III are summarized the data from my cross-compatibility tests. These varieties were intercrossed, each with each, but for the sake of briefness and since nothing would be gained from a detailed account, the data are presented chiefly from the standpoint of the pollen parent.

TABLE III

The pollen of:				
Grimes Golden	used in 213 pollinations	gave	9 sets or	4.2 per cent.
York Imperial	" " 85	" "	15 " "	17.6 per cent.
Stark's Delicious	" " 138	" "	24 " "	17.4 per cent.
Stayman's Winesap	" " 203	" "	9 " "	4.4 per cent.
Ben Davis	" " 131	" "	5 " "	4.5 per cent.

Except for Grimes Golden the per cent. set was increased greatly by cross-pollinations. My results together with those of more than 100 other investigators prove that cross-pollination *does* increase the fruitfulness of an orchard.

Crane and Lawrence ('29) have said: "Sterility in fruits is of three fundamentally different kinds: (1) *generational sterility*, due to the failure of any of the processes concerned with the normal alternation of generations, namely, development of pollen, embryo sac, em-

bryo and endosperm, and the relations of these with one another and with their parents regardless of the cross made; (2) *morphological sterility*, due to the suppression or abortion of sex organs; (3) *incompatibility*."

INCOMPATIBILITY

The above authors, Crane and Lawrence ('29) defined incompatibility thus:

"In this third form of sterility we are not dealing with sterility in the strict sense of the word, as both the ovules and the pollen—or at least a good proportion of them—are functional. The failure to obtain fruits from self- and cross-incompatible pollinations is due to the absence of fertilization, the pollen tubes becoming arrested in the nutrient stylar tissue. On the other hand, in compatible pollinations, although the same pollen and ovules take part, the pollen tubes travel the full length of the style. The male and female nuclei fuse and the fertilized ovary develops into a fruit."

Since Waite's pioneer work on the relative compatibility of pollinations, probably every variety of commercial importance has been tested as to self-compatibility and most of them have been inter-tested as to cross-compatibility.

There are probably a few definite cases of complete physiological self-incompatibility in the apple (examples, Arkansas and Arkansas Black) where the pollen of a variety is not capable of inducing fruit formation in its own ovary.*

This being the case it is only natural to expect that there should be cases of physiological cross-incompatibility. However, Florin ('26), Howlett ('27) and others, agree that no definite cases of physiological cross-incompatibility have ever been found.

The work of East and Mangelsdorf ('25 and '26), Lehmann ('26) and Sirks ('26) all of whom have arrived

* Crane and Lawrence ('30) seem to be in doubt as to the existence of any cases of complete self-incompatibility. They say: "As far as our investigations go, incompatibility occurs in varying degree but is rarely, if ever, completely expressed in apples as it is in plums and cherries."

independently at fundamentally the same conclusions offers a possible theoretical explanation to this problem. Taking, for example, the work of East and his collaborators on the inheritance of self-sterility in highly homozygous tobacco species, they have found that incompatibility is determined by genetic factors just as are morphological characters. These factors form a multiple series, and in a manner similar to some other Mendelian factors any two of them may be carried by a given plant. Pollen can not function in the style of a plant carrying the same incompatibility factors as the pollen. "Like repels like." Self- and cross-pollinations between individuals with the same genetic constitution with respect to incompatibility factors fail.

Following the terminology (Fig. 1) of East and Mangelsdorf ('25) as given by Crane and Lawrence ('29) an individual with the constitution S 1 S 2 can not be fer-

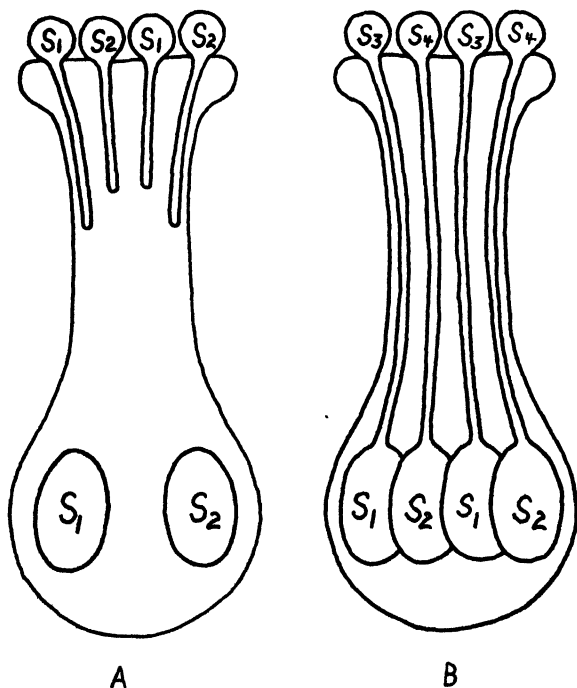


FIG. 1. Explanation in text.

tilized by S 1 or S 2 pollen (Fig. 1A). However, in the cross S 1 S 2 x S 3 S 4 both S 3 and S 4 can effect fertilization (Fig. 1B).

If the work on tobacco holds equally well for apples then the failure to find cases of physiological cross-incompatibility might be explained by the interaction of these sterility factors. Though varieties differ markedly in respect to heterozygosity, some even practically breeding true, the chances are strong that the same factors for sterility will be found in both the male and female gametes on the same plant and hence repel each other. In the case of cross-pollination, even though both parents have factors for sterility, the chances are greater that compatible gametes will have a chance to function. *Cross-pollination* has been proved to increase the fruitfulness of an orchard.

By the interaction of these sterility factors, not only should we expect cross-pollination to bring together favorable gametes but also, due to the highly heterozygous condition of the apple, the gametes produced will be of numerous genotypes and out of a large number of pollen grains there should be some that would be functional on their own ovules. If, then, the stigmas are sufficiently covered with the pollen, even from the same flower, the chances for fertilization are greatly increased. A case in point is the Grimes Golden. I have observed that the styles of this variety which are longer than the stamens bend down as if through some attraction and cover their stigmatic surfaces with pollen from the anthers. As should be expected this variety, although an early bloomer, is a heavy bearer. Most of the self-compatibility tests that have been carried out on this variety have found it self-sterile. Nevertheless, this is one of the most extensively cultivated varieties in the United States and is seldom if ever barren.

Further, as is pointed out in Table IV, "triploid" varieties produce notoriously poor pollen, and neglecting incompatibility the "diploids" as a rule would be more

effective as pollinizers in the field than the "triploids." Crane and Lawrence ('30), however, have shown that "the 'triploid' combinations have in our experiments given even slightly better results in the production of fruits than the 'diploids.' " They account for this in a manner somewhat similar to the case of Grimes above. They say: "Since incompatibility is due to lack of genetic differentiation, the good results obtained from the 'triploids' are probably due to a greater variety in the gametic output of 'triploid' than of 'diploid' varieties, thereby providing a greater chance of compatible combinations." "Triploid" varieties have been shown to be productive in the field, *i.e.*, Bramley's Seedling, Baldwin and Gravenstein. Crane and Lawrence ('30) point out the fact that Bramley's Seedling is the most widely cultivated variety in England. They further add: "In apples, degrees of incompatibility are common even in the so-called 'diploid' varieties; this may be attributed to their secondary polyploid complement (explained below), which involves a polysomic condition of the incompatibility factors. Every chromosome and hence its factors may be represented two or three times in the gametophyte which provides a basis for greater variation in the number of possible combinations of a given factor."

GENERATIONAL STERILITY

As defined by Crane and Lawrence ('29) this form of sterility is due to the failure of any of the processes concerned with the normal alternations of generations; namely development of pollen, etc.

For several years it has been noted that the pollen of certain varieties was, as a rule, functional in producing fruit with most any other variety, if not on the stigma of its own flowers. The results set forth in Table III show clearly that Stark's Delicious pollen is more useful than Stayman's Winesap, for example. Because of this variation in the effectiveness of pollen, it is highly important to know the ability of the pollen of the different

varieties to germinate. I tested the pollen from the varieties that were used in pollination tests on a 5 per cent. sugar medium and found that less than 30 per cent. of the pollen grains of Stayman's Winesap, Baldwin (triploid) and Ben Davis germinated. The pollen of York and Grimes germinated as high as 70 per cent. while that of Stark's Delicious was as high as 90 per cent. Referring to Table III it can be seen that the per cent. germination is correlated with the value of a variety as a pollinizer.

Germination tests have received much attention recently.

Following is a table from Crane and Lawrence ('30).

TABLE IV

Variety	Chromosome numbers ($3n$)				Per cent. pollen germination		
	Rybin	Kobel	Nebel	Darlington and Moffett	Kobel	Kvaale	Florin
Baldwin	48-49	51	51	11.0	12.3	0.0-30.0	
Belle de Boskoop	ca. 46	51	13.0	0.0-30.0			
Blenheim Orange		51	51	0.0-30.0			
Bohnappel	46-49		10.0				
Bramley's Seedling			51	20.9	0.0-30.0		
Crimson Bramley			51				
Damason Reinette	45-47		23.0				
Genet Moyle			51				
Gravenstein	45-46	51	7.0	13.0	0.0-30.0		
Gravenstein (7 clonal varieties)		51					
Harbert's Reinette	45		16.0				
Jaques Lebel	49-51		13.0				
Reinette du Canada	51	38-40	51	4.0	0.0-30.0		
Ribston Pippin	42	51	51	21.4	0.0-30.0		
Roter Eiserapfel	47						
Stäffner Rosenapfel	48-49		25.0	0.0-30.0			
Warner's King	42		27.0	14.8	0.0-30.0		
Winter Zitronensapfel	48-49		21.0	0.0-30.0			
Lane's Prince Albert (1)			34'	57.6	70.0		
Lane's Prince Albert (2)			51				

From this table, which contains only "triploids," except for one case, Lane's Prince Albert, it is seen that all the "triploids" germinated less than 30 per cent. The authors have said, "Although there is considerable variation in the proportion of good pollen among the known 'diploids,' the worst 'diploid' has a much higher proportion than the best 'triploid.' "

However, as pointed out above, even though the pollen from these "triploid" varieties contains a large proportion of non-germinating grains, due to the variation among them they are in most cases valuable as pollenizers.

In some cases the poor germination ability of the pollen has been accounted for from a cytological standpoint. Shoemaker ('25) followed the development of apple pollen from the pollen mother cell stage through to mature pollen. He found that in those varieties, such as Stayman's Winesap, where the pollen is consistently poor, that the reduction division is not regular and a number of pollen grains arise which are unbalanced from the standpoint of chromosomal content and are not capable of germination.

As far as the writer knows, no work has been done on irregularities in development and function of the ovules, nevertheless, no doubt what is said of the pollen grains is probably also true of the female element.

PARTHENOCARPY

To further complicate matters we find that in the apple fertilization apparently supplies the requisite initial stimulus to fruit development and that even one seed need not be the result. Again quoting Crane and Lawrence ('30): "In the apple a single seed is often sufficient for the development of the fruit, and even this seed may be imperfect. This approaches parthenocarp and renders fruit production still less dependent on the formation of seeds. In some varieties of apples entirely seedless fruits are not uncommon." The theory set forth above that the productiveness of "triploid" varie-

ties was due to incompatibility factors being overwhelmed by the increased gametic variation is not strengthened by the fact that Crane and Lawrence ('29) have found only 1.3 good seed per fruit in open pollinated Bramley's Seedling. It is the opinion of the writer that parthenocarpy has not received the attention it should have.

CHROMOSOME NUMBER AND ITS SIGNIFICANCE

According to Crane and Lawrence ('30), 48 varieties have been found to be "diploid" ($2n=34$) and 24 have been found "triploid" ($3n=51$). Hence with an apparent basic number of 17 all the cultivated varieties are orthoploid. Seedlings with an intermediate number (aneuploid) do arise but have been found by Darlington and Moffett ('30) to be of feeble growth and hence would be useless in cultivation.

The "haploid" number of seventeen is an anomaly in the Rosaceae, where seven and eight have been generally found. Darlington and Moffett ('30), in a brilliant contribution, bring forth convincing evidence to support their conclusion that the primary chromosome complement of *Pyrus* is seven and that in the "diploid" *Pyrus* there is a long type of chromosome which is represented four times, while in "triploid" varieties this long chromosome is represented six times. In "diploid" material, morphologically, the 34 chromosomes may be further associated (called multiple association) into seven groups, four quadrivalents and three sexivalents. "*Pyrus* is therefore shown by its chromosome behavior to be functionally a 'diploid' while historically it is quadruply tetrasomic and trebly hexasomic." Further evidence of a primary number of seven is gained from the fact that natural seedlings of "triploid" varieties most frequently have a chromosome number approximating $2n+7$. The genetical complexity of *Pyrus* which the present paper has tried to emphasize is cited as additional evidence.

These findings are both significant and practical, as the authors point out, because of the fact that the balanced condition, as found in the majority of the Rosaceae, has been tested by the rigors of natural selection, while the unbalanced *Pyrus* has yet to put its destiny in the hands of chance.

They say: "Since evolution proceeds largely, if not entirely, by changes in the balance of the hereditary materials, it is plausible that this method of change, the extreme of discontinuity, will in one case out of a very large number yield (at least with later selection) a product as vigorous and as fertile as its antecedents." "It must be remembered that the difference between balance and unbalance is merely the difference between a system that has been tested by natural selection and one that has not. The difference therefore depends upon chances."

I should like to point out the fact, however, that there is little chance of change in the established varieties, due to their method of propagation, and that progress from the above source depends mainly upon the breeding and selection of seedlings.

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THE OPAH OR MOONFISH, *LAMPRIS LUNA*, ON THE COASTS OF CALIFORNIA AND OF HAWAII

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I HAVE previously published two faunal articles on this rare and interesting fish. The earlier (1926) made known its first recorded taking in the Gulf of Mexico. The second (1930) listed the capture of two new (and three old) specimens on our eastern coast and gave a figure of the finely mounted skin of one of these. In this article I also synthesized the natural history of the fish so far as it could be gathered from the widely scattered literature.

In the present article I plan to bring together the scattered published accounts of its occurrence on the Pacific Coast and in Hawaii and to add to these a considerable amount of unpublished data which has come to my hand. Acknowledgment will be made to my informants in the body of the text.

THE OPAH IN CALIFORNIA WATERS

The published records of the occurrence of this fish on the coast of California are very few and very lacking in details. Thus Jordan and Evermann say in 1896, "At Monterey and other places in California. Our specimen is from Monterey." This fish is presumably that also recorded by Evermann the same year (1896). He did not see this specimen (for which no size is recorded) but he easily identified it from the description in a letter sent him. His informant seems to have seen several specimens.

David Starr Jordan in a much later book (1905) said that "the specimen studied by the writer came ashore at Monterey in an injured condition." This possibly is the fish referred to by Jordan and Evermann (1896), and it

may be the one recorded by Evermann (1896) (but on this latter point Evermann writes me, "My impression is that they were two entirely different specimens"). Jordan in the same book (1905) speaks of "another taken at San Pedro Pt. (near San Francisco)."

Very indefinite are Jordan and Starks (1907) who merely say, "Occasionally taken about Santa Catalina. Two stuffed specimens were seen." Later Starks and Morris (1907) say that "Mrs. Andrews, of San Diego, has a painting of this species from a specimen caught in the vicinity. . . . There are two skins of the Opah at Avalon." Next C. F. Holder (1912) states that four or five had been caught near Santa Catalina. He gives two good figures (evidently made from photographs) of the fish (presumably mounted specimens) but nowhere gives any dates of capture, nor any pertinent data as to sizes ("attains a weight of 70 pounds"). This is greatly to be regretted since he probably had practically first-hand knowledge of these specimens.

A more definite note is that of Thompson (1924) on a specimen brought to the San Pedro market in 1924. It was caught about the middle of May, in a mackerel net about one mile off Point Fermin. It weighed about 50 pounds.

The latest published record from the Pacific coast known to me is dated 1929. This (Anon. 1929) is an absurdly written popular (?) account of a 97-pound fish taken in a net off San Pedro. Its "diameter" (dorsio-ventral) is noted as about 3 feet (whether over body only or over dorsal and ventral fins is not stated, but probably the latter) and its thickness as 2.5 inches (which must be an error).

To these few published records of the opah on the southern coast of California are now to be added a considerable number of unpublished ones. First are two which give numerous details, which can be set out in tabulated form. The first is a fish seen and carefully described on October 22, 1918, by Elmer Higgins, of the

U. S. Bureau of Fisheries, who writes of the opah in general that "I distinctly recall seeing several specimens of *Lampris* on the Southern California coast, usually taken in sardine nets which fish in less than 10 fathoms of the surface."

The data referred to were left by Higgins at the California State Fisheries Laboratory at Terminal Island and were copied and preserved by Thompson. Along with these data there is preserved in the same archives the detailed record of another *Lampris* taken off San Pedro and brought to the market on May 12, 1924. For transcripts of these accounts I am indebted to the kindness of Mrs. Genevieve Corwin Wheeler, librarian of the laboratory. These data and those for the Massachusetts fish described in my previous paper are set out in tabulated form below that comparison may be made between the two Pacific fish, and between them and the Atlantic specimen.

COMPARATIVE DATA FOR THREE SPECIMENS OF *Lampris luna*

Measurements in inches	Length, total	Length, standard	Weight, lbs.	Depth	Head, length	Maxillary, length	Diameter of eye	Dorsal, height	Dorsal rays	Pectoral, length	Pectoral rays	Anal rays	Pelvic rays	Depth, caudal peduncle
San Pedro 1918	36	33.5		21.8	11.3	3.6	1.9	9.4	48	9.9	24	42	13	
San Pedro 1924	36.5		46	20	10	3.6	2	10	49	8.5		33	15	2.5
Hyannis, Mass., 1928	31.7	27.1	32	16.7	8.5	2.8	2	10.1	49	8.2	21		14	2

In the table there is but one marked discrepancy, that of the divergent count of anal rays. The commonly accepted count (Jordan and Evermann, 1896) is 38 to 41. Presumably the count of 33 is an error. Additional data for which space is lacking in the table are for the 1918 fish: interorbital space 124 mm (4.9 in.); length of ven-

tral, 266 mm (10.5 in.); pores (*i.e.*, scales?) in lateral line about 80, and in the Massachusetts fish about 86; the caudal rays were 30. Additional data for the 1924 fish are: length of snout, 109 mm (4.3 in.); diameter of pupil 23 mm (0.9 in.).

I will now present certain very fragmentary data for eight other specimens taken around Catalina. These data while very imperfect at any rate indicate the relative abundance of the fish in these waters. All came to me through the kindness of the well-known angler, Mr. Andy Martin, of Beverly Hills, California. None of these data (covering about 30 years) have been previously published. The fish referred to have been mounted, and Mr. Martin has seen every specimen save one. Notice of these will now be set out.

There hangs in the Tuna Club at Avalon, Santa Catalina, a mounted opah measuring 3 feet, 6 inches from tip to tip, and 2 feet, 11 inches in depth (over fins?). Its weight is estimated at about 60 pounds. The fish was taken about 1900. At Avalon, there is another on the wall of Joe Cameron's restaurant, and yet another is on display in MacRae's fishing tackle store. For neither of these latter have I been able to get any measurements or dates of capture.

Two others are on display in large stores in Los Angeles. I have had no answer to my letter concerning the one in the Tuft-Lyons Arms Company. However, the B. H. Dyas Company writes that their specimen is 43 inches (1,092 mm) from tip to tip. The greatest depth is 25 in. (635 mm) and dorsal fin is 8.5 in. (210 mm) high. The dates of capture for these fish can not be ascertained, but both were taken near Catalina.

Three Catalina fish have been mounted by Mrs. C. B. Parker of Avalon, whose letter corroborates Mr. Martin, and have been taken elsewhere. One is in Des Moines, Iowa, and two are the property of Mr. William Wrigley, Jr., of Chicago. No data are available for that at the Chicago Baseball Park, but Mr. Martin has seen and

measured that in the tower room of the Wrigley Building. This mounted fish is 1,270 mm (50 in.) long between perpendiculars; 720 mm (30 in.) deep, and the spread of the tail is 350 mm (14 in.). The depth at the hinder edge of the operculum is 508 mm (20 in.). From the tip of the snout to the center of the eyes is 203 mm (8 in.) and to the hinder edge of the operculum 380 mm (15 in.). The dorsal is 240 mm (9.5 in.) high and 508 mm (20 in.) long. The length of the pectoral fin is 267 mm (10.5 in.), that of the pelvic 267 mm (10.5 in.) and the length of the anal 356 mm (14 in.). The weight is given on the tag as 160 lbs. The fish was taken by market fishermen at Emerald Bay, Catalina Island, in 1924.

In my previous paper it was shown that the opah of our north Atlantic coast is a deep-water fish and is taken ordinarily on trawl hooks. So far as the records go, on the Pacific coast it is taken only on, at, or near the surface, either in nets or with the gaff. Holder wrote in 1912, "It is said that one was taken off San Clemente with rod and reel." This statement has often been repeated and Mr. Wrigley's specimen is also said to have been hooked, but the investigations of Mr. Martin and the officials of the Tuna Club prove these accounts to be erroneous. None on the southern California coast has ever been taken on the hook so far as records go. The universal explanation of their capture at the surface at Santa Catalina is that they get into cold water and become more or less disabled, come to the surface and float about helplessly or feebly swim into bays where the water is warmer—in any case are taken at the surface either in nets or with the gaff.

In my article on the occurrence of the opah on our north Atlantic coast I was able to account for five specimens, or taking into consideration the one on the west coast of Florida, a total of six fish. When one recalls the intensity of fishing operations from Newfoundland to Nantucket, and the length of time they have been carried on, one must draw the conclusion that the fish is very

rare in those waters. Turning, however, to California, there are listed herein six published accounts and two scientific records of its occurrence on the southern coast of California. Of these eight records, two fish were taken at Monterey, one at San Diego and five at San Pedro. There are definite records of eight mounted specimens taken from Catalina waters. There are then sixteen definite records, plus a number of indefinite ones (possibly in some cases duplicates of others) of the occurrence of *Lampris luna* in the waters of southern California. The contrast between these sixteen records for our Pacific coast and the six for our Atlantic and Gulf waters is certainly a remarkable one. Further, since five of these latter were taken between Newfoundland and Cape Cod, that is to say in our northern and colder Atlantic waters, it is particularly notable that on our Pacific coast there are no records for the Washington and Oregon coasts, for the colder Pacific waters.

A FOSSIL MOONFISH FROM SOUTHERN CALIFORNIA

Since the moonfish is now found more abundantly in California waters than elsewhere on our coasts, it is interesting to note that it was found there in geologic times also. Jordan has described (1920) a fossil moonfish three feet long by about two feet deep from beds of Miocene diatoms at Lompoc, Santa Barbara County, California. He concludes his account with the following statement: "The specimen is one of great interest as showing the antiquity of one of the most singular of all living bony fishes, and incidentally with other associated forms, the relative age of the present fish fauna of California."

However, Jordan refers to this fish as "a second species of moonfish." Then he continues, "Two smaller specimens, apparently of the same species, but lacking the head and shoulder girdle, had been previously found at Lompoc." A very indefinite reference led me to find

that Jordan and J. Z. Gilbert in 1919 had, from a study of a portion of the vertebrae only, described these fish as *Diatomoeca zatima* of the family Pleuronectidae. Furthermore this identification is repeated by these same authors in a later publication (1920).

However, Jordan in the *Scientific Monthly* article concludes that these vertebrae, previously determined as pleuronectid, actually belong to *Lampris zatima*. His figure of the nearly complete skeleton leads to the belief that this is correctly identified as a *Lampris*.

Lampris luna IN HAWAIIAN WATERS

This fish is also found in the waters around our island possession. The earliest reference (Jordan, 1905, vol. I, p. 323, fig. 199) is to the giant of the tribe, a specimen in the Honolulu market said to have weighed 317.5 pounds. This figure is made from a photograph sent in by Mr. E. L. Berndt, who captured the fish near Honolulu. Unfortunately Jordan does not give its dimensions or state



—After Jordan, 1905

FIG. 1. An Opah, *Lampris luna*, photographed by E. L. Berndt in the Honolulu Fish Market. Weight listed as 317.5 pounds.

how it was taken. Since this is the record opah of the world, and since it gives the form and markings in photographic reproduction, it is copied here as Fig. 1 to give it a wider distribution than can be had in Dr. Jordan's book.

The next Hawaiian record I find is a very indefinite and unsatisfactory one from Jordan and Evermann (1905), who merely state that "Mr. Berndt sends a photograph of a specimen of this species, weighing 176 pounds, taken off Honolulu." One can not but wonder if this is not the same fish as that referred to above. Had the statements included the dimensions, the matter would have been effectually settled.

Next, Jordan, in the article (1920) on the fossil form previously referred to, figures a beautiful cast of a *Lampris* weighing 100 pounds taken near Honolulu. Whether this is in the Bishop Museum, Honolulu, can not be said. Two years later Jordan and Jordan (1922) in their "Fishes of Hawaii" write that: "An example, six feet long, was once taken at Honolulu. It weighed 217 pounds." This is evidently a repercussion of Jordan's statement in 1905, but inspection of his photographic figure with a tall man standing beside the fish will make it clear that this specimen was not six feet long. These accounts seem to be very much confused.

Finally Jordan and Jordan (1922, p. 29) say that, according to the Honolulu *Star-Bulletin*, another *Lampris* was taken in 1922 about 13 miles west of Oahu at a depth of 1,200 feet. Dr. S. C. Ball, of Yale University, who was at that time connected with the Bishop Museum, writes me that he weighed this fish and that it tipped the scales at 117 pounds. This is the fish referred to by Fowler (1928) as being taken by Japanese fishermen off Waianea beach, Oahu, March 10, 1922. It measured 1,185 mm (46.7 in.).

What is the maximum size of *Lampris luna* can only be conjectured. Jordan says, on what authority I can not

state, that it attains "a length of 6 feet and a weight of 500 or 600 pounds." But it can be stated on the authority of Baikie (1853) that one captured at Sandey, Orkney Islands, was "nearly six feet" long. Now the 317.5-pound specimen figured by Jordan is certainly not that long, and one can only conjecture how much a 6-foot specimen of *Lampris luna* would weigh.

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MCDUGALL'S LAMARCKIAN EXPERIMENT

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A REVOLUTIONARY and important conclusion has been reached on the basis of experimental results by the eminent psychologist, William McDougall.¹ As a result of training rats to perform a specific task for many generations, this investigator claims to have induced inheritance of a specific modification in their behavior. If his data and inferences become established, McDougall will have inaugurated a revolution in genetics even more far-reaching than the one inaugurated by Muller when he increased the rate of mutation in *Drosophila* by means of x-rays. For not only does McDougall claim to have induced mutations, but he claims, in effect, to have induced a progressive series of adaptive mutations. Moreover, a specific treatment was designed to produce just this particular series of mutations and the inference is that any one of many series of mutations might similarly have been produced by appropriate experimental procedures.

McDougall has frequently invited criticism and discussion of his work and, in view of the very great importance of the issue, this seems highly desirable. With this in mind I shall bring together here the impressions the work has made on some other investigators and on myself.

Professor F. A. E. Crew discussed McDougall's work in the *Eugenics Review* for April, 1930. He is of opinion that the work is free from the commoner errors that vitiate most work on the Lamarckian hypothesis, but that it is subject to one very serious flaw: namely, the possi-

¹ McDougall, Wm., "An Experiment for the Testing of the Hypothesis of Lamarck," *The British Journal of Psychology* (General Section), xvii, part 4, April, 1927; "Second Report on a Lamarckian Experiment," *ibid.*, xx, part 3, January, 1930.

bility that the parent rats, which have been trained to the task, communicate to their young something concerning the experience they have been through and that this tradition is built up more and more effectively from generation to generation. So that one deals here not with genetical inheritance but with "social" inheritance.

Professor Crew supports his criticism by an account of similar "social" inheritance among birds. This support for his criticism is really the important thing, because McDougall has already considered and rejected nearly all, if not all, serious criticisms that could be suggested. To Crew's evidence for social inheritance I shall add evidence for two other criticisms that have also been considered and rejected by McDougall. The first of these is the likelihood of inadvertent selection (a criticism so frequently made against work of this sort); the second, and more important, is the possibility that the "improvement" in learning in successive trained generations is an expression not of genetic differences but of differences in the strength of the electric shock used to train the rats.

It will be recalled that the task the rats were trained to perform was to escape from a water tank by means of an unilluminated gangway and invariably to refuse to escape by means of an alternative illuminated one. The method used to discourage the rats from using the illuminated gangway was to electrify it so that each time a rat attempted to use it, the rat would get a shock. Twenty-three generations of rats were trained by means of such shocks to avoid the illuminated gangway. In the later generations the rats required very much less training—that is, fewer shocks—to learn this than they had required in the earlier generations. Although the experiment is still in progress and the published data are merely preliminary reports, McDougall feels that they can only be interpreted as evidences of Lamarckian transmission.

The shocking apparatus was such that, according to McDougall, there was unavoidable variation in the

strength of the primary current, the behavior of the interruptor, and the kind of contact made by the rats. In order to discover whether the difference in mean number of shocks necessary to train the experimental and the control groups of rats might be due to these variations in the strength of the shock, one of McDougall's associates, Dr. Rhine, used three definitely different intensities of shock in attempts to train representatives of the last (23rd) generation of trained rats and of a "control" group (ancestors trained for only four generations). Dr. Rhine found in both groups that the heavier shocks trained more quickly than the lighter shocks. Nevertheless, the rats from the last (23rd) generation of training made a very much better record even when trained by application of light shocks than did rats of the "control" group when trained by application of a heavy shock. In view of the fact that McDougall believes all his shocks to have fallen within the range of medium to heavy, he concludes that variations in the shocks as he applied them could not be very important in determining the mean difference between the "control" rats and the experimental rats. This conclusion seems sound.

These data may be viewed, however, in a somewhat different way. Instead of comparing the records made by the last trained generation with the records of the "controls," the records of the last trained generation when trained by means of the three definitely different intensities of shock by Dr. Rhine may be compared with the records of the same generation when trained by McDougall. Dr. Rhine's results are given in terms of number of days of training required before learning; Professor McDougall's results are given in terms of number of errors made before learning. Either of these data can be converted into the terms of the other with a fair degree of correctness on the assumption (justified by their data) that, before learning, the rats go, on the average, as frequently to the wrong gangway as to the right one. It is known that each rat gets six trials per day. In

Table 1, I have converted Dr. Rhine's data so as to be comparable to the data of McDougall and his assistant, Mr. Heck.

TABLE 1
COMPARISON OF THE RESULTS OF DIFFERENT INVESTIGATORS USING
DIFFERENT INTENSITIES OF SHOCK

Investigator	Dose	Generation	Number of rats	Average number of errors per rat
McDougall	Heavy to medium	23	26	25
Rhine	Heavy	23	4	27
Rhine	Medium	23	4	54
Rhine	Light	23	4	77
Heck	?	14, 15, 16	25	75

Dr. Rhine's data, given in terms of number of days of training required to teach the rats, have been converted by the present author (as explained in the text) into terms of number of errors made by the rats before learning.

This table shows that Dr. Rhine found the mean number of errors (27) made by the rats when trained with heavy shocks to be only half as great as the mean (54) for the same generation of rats when they were trained with medium shocks. Furthermore, the mean found by McDougall (25) is very close to the mean found by Rhine when using the heavy shocks. These two comparisons indicate that McDougall was working at the upper limit of his shocking intensity on this generation and that, had he worked at his lower limit (medium shocks), he probably would have obtained a mean of about 54 errors. A mean of this magnitude is greater than any mean reported for the last seven of the ten generations included in the 1930 paper, with the single exception of the 18th generation with a mean of 62 errors.

I group together the records of the last seven generations to contrast them with the records of the three pre-

ceding generations, for three reasons: First, there is an abrupt change in the records as one passes from the earlier to the later group. The average number of errors for the first three generations (generations 14, 15, and 16) are 80, 70, and 73, respectively. For the next seven generations (with the exception of the 20th generation, for which data are not reported) the averages are 46, 62, 47, 37, 36, and 25, respectively. Secondly, the records of the earlier group of generations do not fall within the range obtained by Dr. Rhine with his medium to heavy shocks, although the records of the later group of generations do (with the single slight exception of the 18th generation, as noted above). Third, the earlier group of generations was trained not by McDougall, but by an assistant, Mr. Heck; the later group of generations was trained by McDougall himself.

There is much to indicate that the marked difference between the records of the generations trained by Mr. Heck and the records of those trained by Professor McDougall may be due not to genetic differences, but to differences in the intensity of shocking. McDougall states that all his own shocks fell within the range medium to heavy; but he makes no statement about the intensity of shock used by Mr. Heck. If we assume that Mr. Heck used a shock corresponding roughly to that designated as light in Dr. Rhine's data, then the means for the three generations trained by Mr. Heck are very close to what would be expected. They are 80, 70, and 73 errors, respectively, yielding a mean of 75 errors for all three generations together. Dr. Rhine's mean for light shock on the last generation (see Table 1) was 77 errors. The surprising agreement between Heck's results on the three earlier generations and Rhine's results with light shock on the very last generation; and the agreement between McDougall's results on the later generations and the results obtained by Rhine with medium and heavy shocks on the last generation, in connection with McDougall's statement that his own shocks varied

within the limits medium to heavy, raise serious doubt as to whether any of the differences between the means of the ten successive generations reported in the later paper (and these are the only generations for which comparable data are available) can be considered as expressions of genetic differences. Indeed, all these differences could equally well, if not better, be interpreted as due to differences in the intensity of shock administered to the rats.

Although it seems to me highly probable that the increase in facility in successive trained generations was largely due to increase in the strength of shock employed, the same result could have been brought about by inadvertent selection. McDougall found that, without differences in antecedent training, there were still differences in learning ability between different rats; and, further, that differences in learning ability were hereditary. Thus, selection of the more apt would result in increase in facility in the course of generations, without any training at all. McDougall's interpretation of his results, therefore, requires that there should be two separate and distinct ways of producing the same result: increase of facility producible by selection of the more apt and the same result producible by inheritance of the effects of training. Since it is known that selection can be effective and it is highly problematical whether inheritance of the effects of training can be effective, it seems possible that the increase in facility found in the experiment may be due to the method known to be effective.

The method employed to prevent favorable selection was to select at random usually two individuals from each litter before training began; these animals were then trained and bred and all litters born after training were equally represented in the selection made for training and breeding in the next generation. One naturally wonders how the two animals selected from each litter were taken at random. Though not mentioned in his published papers, it has been reported that Professor

McDougall stated that this random selection was made by opening the cage and taking the first two animals that came to hand. It has been suggested by an experienced breeder of rats that this procedure made a selection of the most active and clever rats inevitable, because it is always the more active and clever ones that come to the door at once to see what is going on.

There is, moreover, further evidence of selection. In the first place, throughout the experiment, all runts and obviously weakly animals were rejected. Secondly, in the early generations, at least, the presumption is strong that selection was not guarded against as carefully as it was later, because McDougall obtained a new sample of rats for the purpose, as he himself says, of seeing what results would be obtained when *special attention* was given to the matter of selection. This new sample constituted his "control" group. Thus the superiority of the later trained generations to the new "control" stock might well be interpreted as due partly or largely to favorable selection for several generations in the one stock and the careful avoidance of favorable selection from the start in the other stock.

Even in the later trained generations (14th to 23rd) there are several facts which indicate that selection was not completely avoided. In the first place, in each of these generations, as many as three rats were incapacitated by shock from further training and reproduction. Were the rats thus eliminated the worse rats, so far as facility goes? It seems that such must have been the case on the basis of mere chance alone. For, if any particular shock was as likely as any other to result in the incapacitation of a rat, those rats that required most shocks in order to learn would be more likely to get one of these specially severe shocks than those which learned quickly. Obviously, rats which learned after three shocks would have much less chance of receiving such an unfortunate shock than those which required three hundred shocks. It seems probable that the rats eliminated

in each generation by fatal shocks were, on the average, the worse rats.

Furthermore, there is clear evidence that not all the individuals trained in any one generation were represented by descendants in the next generation. From the data presented by McDougall, there is no way of telling whether the ones thus eliminated were the worse ones, the better ones, or intermediate ones. McDougall merely states that "all litters born of trained parents were equally represented in the next generation." The question then arises: did all trained parents produce litters? This question can be definitely answered for the 11th, 13th, and 21st generations. In order for every individual to be represented by at least one descendant, the number of individuals trained in the parent generation must have been not more than twice the number trained in the next generation. For, if the sexes are equally represented among the parents, then the least number of matings possible which includes all parents is half the number of the parents; so that the least number of litters is also half the number of the parents, and if only one individual is selected from each litter, the least number of offspring is also half the number of the parents. If the sexes were not equally represented in the parent generation, there would have to be a correspondingly greater number of matings, litters and offspring for each parent to be represented by at least one descendant. McDougall does not state the distribution of the sexes in his groups, but granting the best possible case, namely an equal representation of the sexes and the representation of each parent by only one descendant, it is still possible to demonstrate that some trained parents must have been unrepresented by descendants. Thus, there were 41 animals trained in the 11th generation and only 16 in the 12th generation. So that, at the very best, not more than 32 of the 41 animals trained in the 11th generation were represented by progeny. Similarly, from the facts that 23 animals were trained in the 13th generation and 10 in the 14th generation, it can be stated with certainty that

at least three of the 23 animals trained in the 13th generation were not represented by progeny. Likewise, it can be shown that at least 2 of the 34 rats trained in the 21st generation were not represented by progeny.

The preceding calculations are based on the assumption that only one individual from each litter was trained. McDougall states that usually two individuals from each litter were trained. If two individuals were trained, then the number of trained rats in any generation should at least equal the number trained in the preceding generation in order to give equal representation to all trained parents. On this basis, at least 25 of the 41 animals of the 11th generation, 13 of the 23 animals of the 13th generation, 4 of the 10 animals of the 15th generation, 6 of the 22 animals of the 18th generation, and 18 of the 34 animals of the 21st generation were not represented by progeny.

Thus, it is definitely certain that some trained individuals of some generations were not represented by trained descendants and it is highly probable that many more were not. The question of importance is: Which ones were not represented? McDougall states that either the individuals to be bred were chosen before training began or the best and worst rats of each litter were selected to be parents of the next generation. Nevertheless, it is highly desirable to know what the records of the previously chosen rats actually turned out to be. In the major experiment, pedigrees are not given so that it remains uncertain as to how great a part inadvertent selection played.

Some light on the importance of selection may be expected in McDougall's next report, because he is now conducting what promises to be an illuminating experiment. He is deliberately selecting the worse half of the animals in each generation to be the parents of the next generation. As he quite justly feels, a marked increase in facility in successive generations even against strongly unfavorable selection will be strong evidence for his claims of Lamarckian transmission.

As the work now stands, however, there is one point (already briefly mentioned above) that requires further consideration, namely the very great difference between the later trained generations and the "controls." It has already been pointed out that selection among early generations of trained rats and careful avoidance of selection among "controls" might account for much of this difference (see page 547). McDougall ascribes this, however, to the effect of many generations of training, on the one hand, as opposed to few generations of training, on the other hand. This conclusion is based on the assumption that the "control" and experimental stocks were genetically similar with respect to the characteristics upon which facility in their task depends. In support of this assumption there is only the fact that the control stock was obtained, as was the experimental stock, from the Wistar Institute Standard Inbred Stock. The former, however, were obtained in 1927, the latter, in 1920. For seven years the two stocks were bred hundreds of miles apart.

It is, of course, not at all clear that the two stocks were genetically identical with respect to the characteristics in question, even in 1920. McDougall found large differences between individuals and between families in these respects in the early generations of his experimental stock. There are, moreover, no data for the early generations of the experimental stock that are strictly comparable with those obtained seven years later in the "control" stock. The conclusions drawn from a comparison of these two stocks are thus subject to serious criticism.

In the continuation of his work it is to be hoped that Professor McDougall will be able to give a definite experimental answer to each of the alternative interpretations that have been suggested by his critics. Happily, each of these criticisms is susceptible of definite experimental test. Only when these have been made can his very important conclusions be considered as fully borne out by his evidence.

SHORTER ARTICLES AND DISCUSSION

PARASYNAPSIS AND APPARENT CHIASMA FORMATION IN *OENOTHERA*

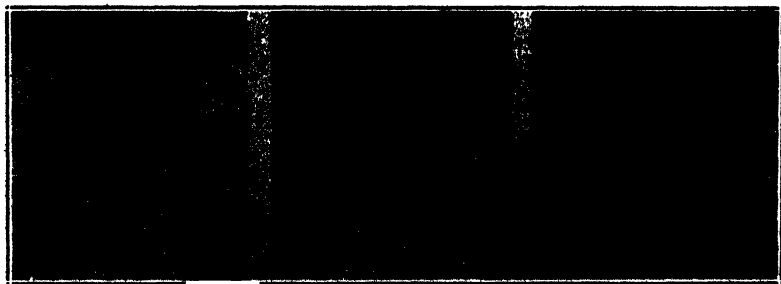
THE behavior of the chromosomes of *Oenothera* during the second contraction in the prophase of meiosis is rather obscure. Various authors (Cleland, 1922, 1924, etc.; Håkansson, 1928; Weier, 1930; Leliveld, 1931; and others) agree that the chromatic threads thicken greatly during this contraction from which the fully formed "diakinesis" chromosomes eventually emerge. During this process the identity of the chromosomes as individuals is obscured by the massing of threads. It is believed by some authors (Håkansson, 1926; Cleland, 1926; Illick, 1929) that the two sides of the loops thrown out from the second contraction knot may represent side-by-side pairing of homologous (parts of) chromosomes, but it is impossible from the figures seen to determine the extent of individual chromosomes. My own observations on various species of *Oenothera* agree with those of the other investigators except for the case to be described.

In a permanent smear preparation (Nawaschin, crystal violet) of a single bud of an *Oenothera* species not yet described, the microspore mother cells were in various stages ranging from early second contraction through late "diakinesis." The diakinesis figures showed that this plant had seven free pairs of chromosomes. In six cells from a single pollen sac the chromosomes, which were in a comparatively thin thread-like stage, were more or less evenly distributed throughout the nucleus so that they appeared distinct and separate. These chromosomes showed a marked side-by-side pairing. They strongly resembled typical parasynaptic chromosomes of a corresponding stage in other organisms. Chromosomes of these six nuclei are illustrated in Figs. 1 to 6 and in the photomicrographs.

There was some evidence (see especially Fig. 1-a) that the chromosomes were in the four-strand stage. Such appearances are difficult to illustrate accurately, and I am not convinced that the figures conclusively showed this condition. Appearances of chiasmata were evident, but it seems impossible in this material to be sure that they actually are true chiasmata (i.e., a cross



FIGS. 1 to 6. Camera lucida drawings ($\times 2600$) of chromosomes of six nuclei showing parasynaptic conjugation.



FIGS. 7 and 8. Photomicrographs of the nucleus drawn in Fig. 2.
 FIG. 9. Photomicrograph of some of the chromosomes drawn in Fig. 1
 (all $\times 880$).

between two of four strands). In another cell of the same pollen sac appearances of chiasmata were noted, but in this case the chromosomes were much thinner and longer and it was impossible to determine the extent of any chromosome pair.

The six nuclei referred to were in a stage generally included in late second contraction. The features described for these cells are not ordinarily discernible because of the massing of the threads about the nucleolus. It is probable that these features occur commonly in all *Oenotheras* and were seen distinctly in this case only because the preparation was unusually favorable.

In the published literature of the *Oenotheras* there is a single figure that shows the features described here (Håkansson, 1928, Fig. 2-d). This figure represents a megaspore mother cell of *Oe. Lamarckiana*, which had a ring of 12 chromosomes and one free pair. The free pair shows strong side-by-side pairing with appearances of chiasmata. Certain arms of the ring chromosomes which project from the knot illustrate a similar condition which by analogy may be taken to represent parasynaptic pairing of the homologous regions of the chromosomes constituting the ring. This interpretation was tentatively advanced by Håkansson.

Håkansson (*loc. cit.*) found seven to be the maximum number of loops thrown out from the second contraction knot in the megaspore mother cells of *Oe. Lamarckiana*, while Weier (1930) found thirteen such loops in the pollen mother cells of the same species. Both authors conclude that the constriction between two adjacent chromosomes of the ring should occur approximately in the middle of the loop. It seems to me that one should not expect to find a constant number of such loops in these figures, since some of the loops should be included in the relatively large amount of chromatin in the tighter part of the knot. If one could obtain figures of *Lamarckiana* showing all the chromosomes as distinctly as they were seen in figures of another species reported in this paper, he might expect to find a star-shaped structure with twelve arms and a free pair (see discussion of Belling's translocation hypothesis in Darlington, 1929, and in Emerson, 1931a). Genetical findings have shown that crossing over occurs both in pairing chromosomes and in chromosomes in large rings (Shull, 1923a, b; Emerson, 1931a, b). It follows, on the assumption that chiasma formation and crossing over are related phenomena, that appearances of chiasmata

should be found in arms of the ring chromosomes (during second contraction) as well as in the pairing chromosomes. The figure of *Oe. Lamarckiana* found by Håkansson (referred to above) is in agreement with this expectation.

In connection with such rarely observed parasynapsis in *Oenothera*, it should be remembered that Schwemmle (1926) found indications of parasynapsis in one series of preparations in *Eucharidium* in which the various processes connected with reduction progressed slowly, while in another series, in which the process was more rapid, only the "telosynaptic" appearances commonly found in *Oenothera* were observed.

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CHROMOSOME STRUCTURE IN *DROSOPHILA*¹

THE findings of Muller and Painter (1), Painter and Muller (2), and Dobzhansky (3, 4, 5) to the effect that discrepancies occur between the genetical and cytological maps of *Drosophila melanogaster*, recommends a critical study of the organization of the chromosomes of that genus. The present writer has applied, therefore, to *Drosophila* those methods found satisfactory for the preservation of the more intimate details of the structure of plant chromosomes (6). Two species of *Drosophila* have been studied, *D. melanogaster* and *D. virilis*. Chromosomes of somatic and meiotic mitoses have been investigated, but especial attention has been directed to the organization of the spiremes in such large nuclei as those of the salivary glands. The present report will be followed by a more detailed account.

Well-fixed somatic nuclei show the spiremes to be composed of two optically differentiated substances, designated as chromatic and achromatic. The latter stains faintly with iron hematoxylin; the former is, by contrast, hematoxylin avid. Many preparations reveal the spireme as apparently discoid, with alternating bands of chromatic and achromatic material. A similar observation has recently been made by Kostoff (7) in *D. melanogaster*. Although the chromatic cross-striations often appear as single bands, favorable technique reveals that this aspect is due to the close approximation or lateral fusion of a pair of narrow threads. Both single and double bands are portrayed in Figs. 1A and 1D, the former a portion of the spireme of *D. virilis*, the latter of *D. melanogaster*. In other preparations, or even in other por-

¹ Presented to the Biological Section of the Alabama Academy of Sciences, March 13, 1931.

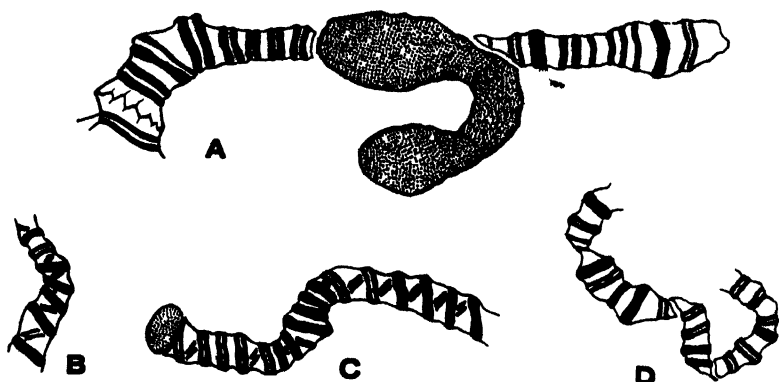


FIG. 1. Structure of the spireme in *Drosophila*. A—indications of cross-striations in *D. virilis*; B—chromonemata in *D. melanogaster*; C—chromonemata in *D. virilis*; D—cross-striations in *D. melanogaster*. B and C are regarded as presenting a more accurate picture of the normal structure than A and D. All from camera-lucida drawings.

tions of some nuclei in which the discoid appearance is observed, the continuity of the chromatic material is evident, in the form of parallel spiral bands. These are embedded peripherally in the achromatic matrix. Figs. 1B and 1C illustrate such organization in portions of the spiremes of *D. melanogaster* and *D. virilis*, respectively. It will be noted in both of the figures that the double spirals are fused in places, to give the appearance of a single thread. Because of the continuity which they maintain, the chromonemata are assumed to represent the gene-carrying material, a possibility suggested by Muller in 1916 (8).

The probable relationship of cross-striations to chromonemata has been discussed extensively by several writers, particularly in reference to the spireme in the salivary glands of the dipteran, *Chironomus*, and to the arrangement of the chromatic material in the monocotyledon, *Tradescantia*. Without renewing the arguments presented elsewhere (9), it is the opinion of the writer, based on a critical study of the chromonemata in plant chromosomes, as well as in *Drosophila*, that the appearance of the discoid or discontinuous structure is the expression of an inadequate technique, the spiral bands presenting a more accurate picture of the normal arrangement of the chromatic material.

It has not been possible to trace the chromonemata through the entire mitosis, as the writer has done in several plants, because the more compact metaphase and anaphase chromosomes of

Drosophila do not reveal distinctly their internal structure. However, the moniliform outline, and a median vacuolar region occasionally seen in these chromosomes, suggest the presence of spiral bands. Such intimations of internal organization are commonly viewed in metaphase and anaphase plant chromosomes, although more favorable preparations disclose the chromonemata at these stages.

The presence of chromonemata in *Drosophila* may aid in explaining the discrepancies between the genetical and the cytological maps. If, at the time of crossing-over, when the genetical maps are determined, the chromonemata are entirely extended, or nearly so, and if in the more compact chromosomes, from which the cytological maps are determined, the chromonemata are tightly or unevenly coiled, there is presented a mechanism which permits the maintenance of the linear order of the genes and at the same time allows considerable variation in the scale of their distribution from one region of the chromosome to another. Translocations, as seen in metaphase plates, could therefore be of slight size visibly and yet contain a relatively large portion of a very tightly coiled thread. Conversely, a larger fragment with a loosely wound or extended thread, might represent only a few units genetically. Certainly the coils, where discernible, vary considerably in pitch from one region to another. This factor, coupled with those variations in the diameter of the chromosome, and consequently in the helix during different turns (see Fig. 1), suggests the possibility of considerable variation between measurements made along the chromonemata and those made along the greater axis of the chromosome.

Dobzhansky (4), commenting on the fact that the third chromosome of *D. melanogaster*, when seen cytologically, appears thinner at the middle and rather thickened at the ends, states, "This may suggest that the distances between the genes located far from the spindle fiber are shorter when the chromosome is in the metaphase-plate stage than they are in the stage when crossing-over takes place." Whether the discrepancy between genetical and cytological maps is too great to be accounted for by the different diameters of the different parts of the third chromosome, as Dobzhansky (3) concludes, remains to be investigated in the distribution of the chromonemata in that chromosome.

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THE MULTIPLE SOMATIC EFFECTS OF THE BAR GENE IN *DROSOPHILA MELANOGASTER*¹

INTRODUCTION

As a result of the extensive studies on the character "bar" eye of *Drosophila*, carried on in this laboratory, and the tendency which was noted for this stock to revert to full or mutate to ultra-bar, the question arose as to the exact extent of the somatic changes produced by a change in this gene. When the gene that produces the bar eye is lost, or doubled, what other parts of the body are correlated with this change? What are the multiple effects of the single gene?

Grateful acknowledgment is here made to Dr. Charles Zeleny, of the University of Illinois, for his suggestions and for the opportunity to work on this problem under his guidance; to Dr. David H. Thompson, of the Natural History Survey, for helpful criticisms and permission to use unpublished data; to Dr. A. H. Sturtevant, of the California Institute of Technology, for the bar stocks used in the experiment (since the writer was in California during part of the course of selection of the stocks); and to Dr. Theodor Dobzhansky for the forked bar and the forked ultra-bar which he had derived from it for his own experiments.

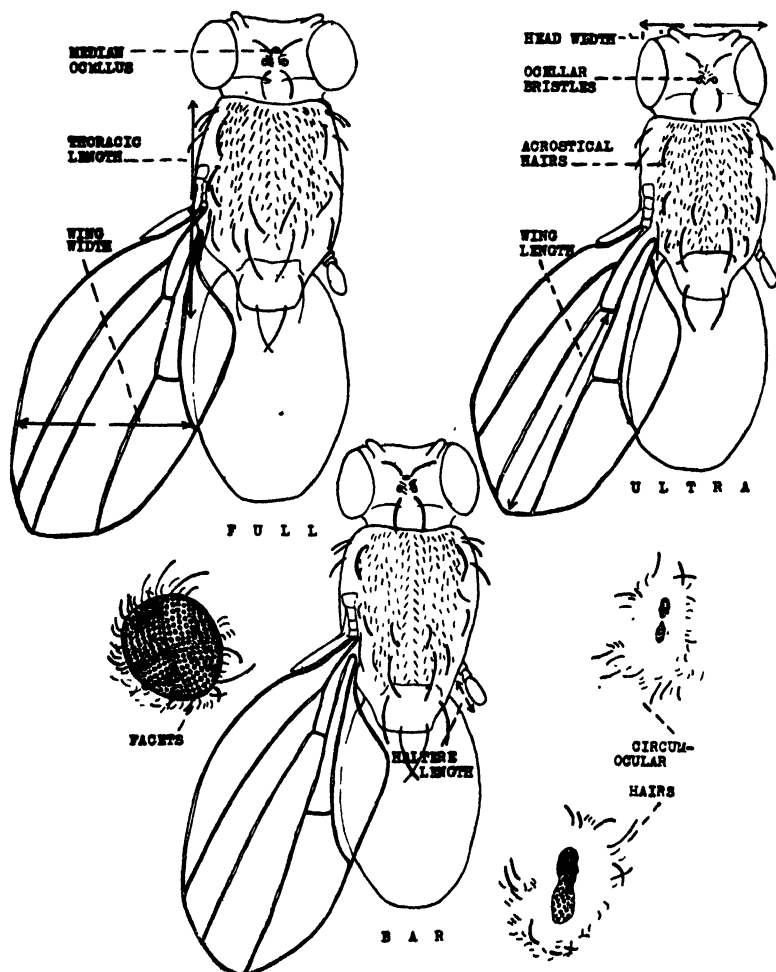
MATERIALS AND METHODS

The material used in this investigation consisted of two strains of red-eyed bar flies—one with straight bristles and one in which the bristles were forked. From each strain, mutations to ultra-bar and reversions to the full or normal-eyed condition were

¹ Contribution from the Zoological Laboratory of the University of Illinois, No. 397.

obtained, and stocks established so that it was possible to check the results obtained with one series against the other.

The problem under discussion is concerned only with the somatic effects and no effort was made to demonstrate any physiological relationship between the bar genes and the condition of ultra-bar (which Sturtevant calls "double-bar") or reverted full, which is attributed to the absence of the bar gene. The somatic determinations were checked against facet count, and consisted of measurements of different parts of the body—such as head width, wing length and width, haltere length; bristle counts of rows of acrostical hairs, circumocular and ocellar hairs; also a



determination of the proportionate size of the median ocellus, which fluctuates as compared with the two lateral ocelli which do not change.

While it is obviously impossible to eliminate *all* the modifying factors, or to prevent new modifiers from arising during the course of an experiment, the stocks were rendered as homogeneous as possible by inbreeding for the bar strains, and by taking the most recent mutations or reversions for the ultra and full-eyed groups.

Parent flies were bred at 25° C. and the experimental offspring from such parents were isolated, as they emerged, and made up in pair matings for each eight-dram vial for the full and bar-eyed strains; with two-pair matings for the ultra-bar because of its lower fertility. An effort was made to have all the flies used of the same age, thereby minimizing the factor of age. The flies were placed in the 27° incubator after they had been transferred to fresh food, the media used being banana-agar with a pinch of dry Yeast Foam; and as soon as the number of eggs laid in any one vial approached twenty, the parents were removed. More than twenty flies in this size vial results in overcrowding and unsatisfactory food conditions for the larva (as shown by Eigenbrodt and others); while fewer than that number are unable to keep down the growth of the yeast long enough for their own developmental period.

As soon as the larvae had pupated and new flies hatched out, they were isolated and preserved in 75 per cent. alcohol. All counts and measurements were made on flies which had been so preserved.

In all, 100 flies were counted from each stock, 50 males and 50 females, and the averages given are the results of these measurements.

The possible source of error due to the development of accessory modifiers during the course of the experiment has been mentioned. The temperature was controlled automatically and was subject to a change of less than 0.5° C. in either direction from 27°. In order to reduce the personal error, recounts and checks were made on a large number of the measurements.

RESULTS

The results of this study are summarized in the accompanying tables, which have been arranged so that a comparison is made

between the females of the straight and forked strains, and in like manner the males—instead of comparing the males with the females in each group, as there is a marked sexual dimorphism.

Since the original difference observed between the several strains was the number of facets each possessed, facet count has been taken as the basis for comparison. Full, bar and ultra flies are sharply marked off from each other, with no overlapping in facet number. The facet count of the females is noticeably less than that of the males. The forked strain closely approaches the straight-bristled stock in this respect.

Correlated with facet count is the width of the head, since the entire head width is measured and this is increased by the size of the compound eyes in full. The full-eyed flies of both sexes had a much greater head width than the bar or ultra-bar flies.

TABLE I
AVERAGES BASED ON MEASUREMENTS OF 100 FLIES—50 MALES AND 50
FEMALES FROM EACH STOCK
(In units of 18.4 microns)

	Facet counts	Head width	Haltere length	Thoracic length	Wing width
♀ ♀					
Full	700	47.88 ± .17	14.12 ± .06	56.56 ± .15	51.70 ± .14
Bar	65.18 ± .71	37.92 ± .15	13.84 ± .10	53.52 ± .28	51.16 ± .21
Ultra	16.84 ± .35	34.66 ± .10	13.46 ± .08	49.38 ± .32	48.82 ± .21
Forked Full	700	45.92 ± .14	14.14 ± .07	54.94 ± .24	51.30 ± .11
“ Bar	62.98 ± .66	35.54 ± .11	13.56 ± .10	53.22 ± .16	49.94 ± .16
“ Ultra	17.30 ± .38	34.62 ± .16	13.00 ± .07	49.88 ± .22	47.60 ± .24
♂ ♂					
Full	750	43.72 ± 1.60	13.82 ± .07	49.14 ± .01	45.80 ± .13
Bar	99.30 ± .81	35.16 ± .13	13.56 ± .06	46.94 ± .22	44.88 ± .18
Ultra	18.78 ± .20	32.20 ± .09	12.54 ± .13	44.46 ± .22	43.80 ± .15
Forked Full	750	42.64 ± 1.47	13.74 ± .09	48.92 ± .26	45.74 ± .13
“ Bar	87.44 ± .09	34.02 ± .11	13.14 ± .05	46.60 ± .20	44.70 ± .16
“ Ultra	19.78 ± .38	32.18 ± .11	12.66 ± .09	45.50 ± .16	43.72 ± .16

The haltere length of bar again places it in an intermediate position between full and ultra, although the forked and straight-bristled strains are nearer together here than in any other single measurement.

Sexual dimorphism is shown by a comparison of the size of the males and females of all groups. Particularly does thoracic length contribute to making the females much larger than the males. Correlated with the thoracic length is the size of the wings—especially the wing length.

Wing width was measured to include its greatest diameter from the Vth longitudinal vein to a point directly across and at right angles to its length. The wing length was measured from the anterior cross-vein to the tip of the IIIrd longitudinal.

TABLE II
AVERAGES BASED ON MEASUREMENTS OF 100 FLIES—50 MALES AND 50
FEMALES FROM EACH STOCK

	Wing length	Ocellar hair number	Circum- ocular hairs	Acrostical hair number	Size of median ocellus
♀ ♀					
Full	76.24	6.26	53.90	16.54	.93
	± .22	± .04	± .14	± .10	
Bar	73.62	6.88	49.86	17.60	.73
	± .30	± .09	± .29	± .11	
Ultra	72.62	7.11	45.40	18.06	.05
	± .32	± .11	± .31	± .07	
Forked Full	76.20	5.84	43.10	15.94	.91
	± .18	± .08	± .13	± .07	
“ Bar	73.40	6.30	39.16	16.24	.74
	± .32	± .06	± .15	± .13	
“ Ultra	72.56	6.68	36.66	16.58	.12
	± .30	± .13	± .28	± .58	
♂ ♂					
Full	67.12	5.88	52.14	16.28	.82
	± .20	± .05	± .11	± .05	
Bar	65.28	6.30	47.78	17.12	.68
	± .30	± .05	± .26	± .10	
Ultra	64.20	6.88	43.12	18.02	.95
	± .29	± .11	± .28	± .07	
Forked Full	67.12	5.88	40.96	15.96	.8
	± .14	± .08	± .13	± .07	
“ Bar	65.20	6.20	38.28	16.22	.4
	± .35	± .06	± .18	± .05	
“ Ultra	64.48	6.92	34.96	16.24	.8
	± .17	± .11	± .24	± .04	

The place of the missing ocellus in ultra-bar is not infrequently taken by one or more ocellar hairs; and the number of the latter stands in inverse relationship to the number of facets present.

Essentially the same thing is shown, though to a less striking degree, by the number of rows of acrostical hairs found in the different stocks; while the reverse holds true for the rows of circumocular hairs immediately surrounding the compound eye.

The size of the median ocellus is very closely associated with the factor for bar, so that one can almost tell by *its* size alone to what strain the fly possessing it belongs, since it is largest in full and smallest or even altogether lacking in ultra. The lateral ocelli do not change in size and were used as the basis for comparison, the median ocellus being understood to be in a percentage ratio to the lateral ocellus next to it.

CONCLUSIONS

The basis for an interesting speculation as to the extent of the developmental effects of single genes is provided—when the single gene for “bar” produces so marked a change as the reduction in facet count from 700 or more in full to less than a hundred in bar.

The single striking change is in the inhibition of the facet-forming substance, but correlated with it are other less marked, but perfectly definite and consistent somatic changes. As might be expected, these are most pronounced in the head region and in association with the eye itself; the width of the head, which is directly affected by the size of the eye, the size of the median ocellus and the number of ocellar hairs.

One of the criticisms made of the mutation theory of evolution is that it is inconceivable that there should be proper physiological correlation of organs if each part has arisen by an independent mutation. This is based upon a misconception of the extent of the somatic effects produced by a single gene change, for, as may be seen from the data herewith presented, even where one part is especially modified, other effects are evident in a great many parts of the body.

In this case the one known difference between the stocks was the factor governing facet-formation, and evidently the factor which places the bar stock in an intermediate position between full and ultra exerts a similar influence on practically all measurable parts of the body. “Full” predominates in everything

except the ocellar bristle number and the number of rows of acrostical hairs. In these latter instances the relationship is reversed, although bar still retains its intermediate place.

Incidentally, in working with the forked and straight-bristled strains certain differences were noted between them.

The conclusions to be drawn from these observations may be summed up as follows: (1) The somatic effect of the bar gene is primarily a reduction in facet number; (2) when this effect is doubled, as in ultra-bar, the facet number is again appreciably reduced; (3) head width, haltere length, thoracic length, wing length and width, and size of the median ocellus are directly correlated with the facet count; (4) while the number of rows of acrostical hairs and the ocellar hair number are in inverse relationship; (5) in almost every respect the forked bristled flies are smaller than those with the straight bristles; (6) proof of the wide-spread somatic effects produced by a single gene change is shown by the fact that observations made upon either the forked or straight-bristled strain are substantiated by similar observations upon the other.

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EXPERIMENTALLY INDUCED ALTERATIONS OF THE MORPHOLOGY OF CHROMOSOMES

THE recent experiments with X-radiations (Painter and Muller 1929, Dobzhansky 1929) show that striking alterations in the morphology of chromosomes are obtainable in this way. An analogous treatment was also applied in the Cytological Laboratory of the Institute of Plant Industry (Leningrad) to the seedlings of *Secale cereale*, *Crepis capillaris* and to some other plants with similar results. Fragmentations, losses of chromosome parts, and translocations of parts from one chromosome to another were observed some days after treatment. Three figures selected from among numerous observed conformations may serve as illustration.

In a plate of *Secale cereale* (Fig. 1) two out of 14 chromosomes—consisting normally of two well-developed arms—are provided only with oval heads, which probably represent the remaining parts of their respective arms, the major parts of the latter being lost.



FIG. 1. *Secale cereale*. Nuclear plate from a seedling treated with x-rays. Two chromosomes are provided with heads.

In one plate of a treated *Crepis capillaris* (Fig. 2) chromosome A' shows a newly formed secondary constriction, chromosome D is deprived of the greater part of its large arm, and on the contrary chromosome C' is considerably lengthened—evidently at the expense of the translocated part of chromosome D.



FIG. 2. *Crepis capillaris* Wallr. Nuclear plate of a seedling treated with x-rays. The constrictions are achromatic.

In a second plate of the same species (Fig. 3) the long arm of chromosome A is fragmented. Chromosome A' is fragmented at its constriction. Both separated arms have formed little heads and constrictions and so have turned into two new types of normally constituted chromosomes.

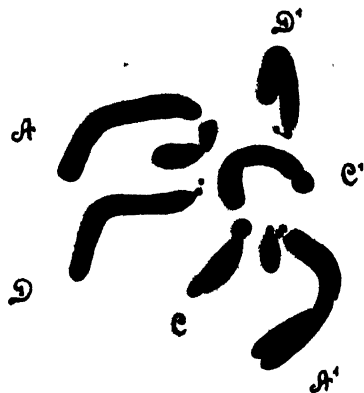


FIG. 3. *Crepis capillaris* Wallr. Nuclear plate of a seedling treated with x-rays.

There is no doubt that various other agents can produce similar effects—as we have found by means of alcohol treatment. The most interesting alteration we have obtained is represented in Fig. 4. Chromosome D' has augmented its head but lost its satellite, chromosome C' on the contrary has diminished its head but acquired a satellite.



FIG. 4. *Crepis capillaris* Wallr. Nuclear plate of a seedling treated with dilute alcohol.

The chromosome changes described above happen under apparently normal conditions as well. A number of diverse conformations have been observed by us in an individual of *Crepis tectorum*. A rather complex case is represented in Fig. 5. One sees here 10 chromosomes instead of 8—some of them being short fragments, which are nevertheless provided with constrictions and heads.

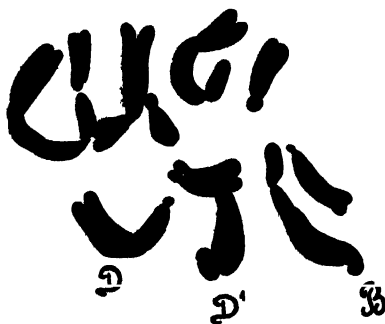


FIG. 5. *Crepis tectorum* L. Altered nuclear plate from a plant grown under apparently normal conditions.

It therefore seems quite reasonable to suppose that large and sudden alterations of chromosome morphology can take place in the process of evolution.

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THE EFFICIENCY OF THE CORRELATION COEFFICIENT FOR ESTIMATING LINKAGE INTENSITIES

A SEARCH for a rapid and reliable method for estimating linkage intensities led Y. Takezaki (6) in 1925 to propose a formula based on the method of treating the fourfold table of phenotypic frequencies as a correlation table. Working quite independently, F. V. Owen (5) developed the same method in 1928. Both authors presented tables to facilitate the rapid calculation of linkages by this method.

In the F_2 generation of a cross between parents with two factor pairs differing there are to be expected, normally, four classes of zygotes. These may be designated AB, Ab, aB and ab. If the number of individuals obtained in each of these four classes is designated as a, b, c and d, respectively, and the total number observed as n, the value of r^2 (derived originally for

the fourfold table by Boas (1), Johannsen (4) and Yule (7) is given by

$$r^2 = \frac{(ad - bc)^2}{(a+b)(c+d)(a+c)(b+d)} \quad \text{I}$$

For a , b , c and d we may now substitute the values of their expectations in terms of the proportion, p , of the AB and ab gametes, namely

$$\frac{n}{4}(2+p^2, 1-p^2, 1-p^2, p^2)$$

We then have

$$r^2 = \frac{(4p^2 - 1)^2}{9} \quad \text{II}$$

From whence

$$p = \frac{1}{2} \sqrt{3r + 1} \quad \text{III}$$

if r is taken to be positive when ad exceeds bc . This is the formula derived by Takezaki (6) and Owen (5).

The cross-over percentage, expressed as a decimal fraction, will then be given directly by p when crosses are made in the repulsion phase and by $1-p$ when made in the coupling phase.

Takezaki derived a formula for the standard error of his estimate of p from the assumption that the standard error of r , obtained from the fourfold table by equation II, could be equated to the standard error of a correlation coefficient derived from a normal frequency surface having the same number of observations. This mistaken assumption has led to the precision of this method of estimating linkages being greatly overestimated. On Takezaki's assumption the standard error of his estimate of p may be calculated as follows:

The variance of r (V_r) from a product moment correlation coefficient is $\frac{(1-r^2)^2}{n}$

$$V(p^2) = \frac{9}{16} V(r)$$

Then

$$V(p^2) = \frac{9(1-r^2)^2}{16n}$$

To obtain the variance of p we divide the variance of p^2 by $4p^2$

$$\text{or} \quad V(p) = \frac{9(1-r^2)^2}{64p^2n}$$

And the standard error of p is

$$\frac{3(1-r^2)}{8p\sqrt{n}} \quad \text{IV}$$

Fisher (3, p. 249) has given a general method for calculating the sampling variance (standard deviation squared) and thence the standard error of any estimate expressible explicitly in terms of the frequencies. The method involves the differential coefficients of the function in question with respect to each observed frequency and to the total, n . Applying this method to the problem of deriving the true standard error of p we proceed as follows:

$$V(r) = V \left\{ \frac{ad - bc}{\sqrt{(a+b)(a+c)(b+d)(c+d)}} \right\}$$

Substituting in the general equation (3)

$$\frac{1}{n} V(r) = S \left\{ p \left(\frac{\partial r}{\partial a} \right)^2 \right\} - \left(\frac{\partial r}{\partial n} \right)^2 \quad V$$

where r is the value calculated from the fourfold table and p is here, for each class of zygotes, the probability of an F_2 individual falling in that class. Differentiating, we obtain

$$\frac{\partial r}{\partial a} = \frac{d}{\sqrt{(a+b)(a+c)(b+d)(c+d)}} - \left\{ \frac{ad - bc}{(a+b)(a+c)(b+d)(c+d)} \cdot \frac{(a+b+a+c)(b+d)(c+d)}{2\sqrt{(a+b)(a+c)(b+d)(c+d)}} \right\}$$

$$= \frac{d}{\sqrt{(a+b)(a+c)(b+d)(c+d)}} - \frac{1}{2} r \left(\frac{1}{a+b} + \frac{1}{a+c} \right) \quad VI$$

$$\frac{\partial r}{\partial b} = \frac{-c}{\sqrt{(a+b)(a+c)(b+d)(c+d)}} - \frac{1}{2} r \left(\frac{1}{a+b} + \frac{1}{b+d} \right) \quad VII$$

$$\frac{\partial r}{\partial c} = \frac{-b}{\sqrt{(a+b)(a+c)(b+d)(c+d)}} - \frac{1}{2} r \left(\frac{1}{a+c} + \frac{1}{b+d} \right) \quad VIII$$

$$\frac{\partial r}{\partial d} = \frac{a}{\sqrt{(a+b)(a+c)(b+d)(c+d)}} - \frac{1}{2} r \left(\frac{1}{b+d} + \frac{1}{c+d} \right) \quad IX$$

Since the expected frequencies in the four classes are equal to

$$n \left\{ \frac{2+\theta}{4}, \frac{1-\theta}{4}, \frac{1-\theta}{4} \text{ and } \frac{\theta}{4} \right\},$$

where $\theta = p^2$, we now substitute these values for a, b, c and d in equations VI, VII, VIII and IX, respectively, and obtain

$$\frac{4\theta}{3n} - \frac{2}{n} \left(\frac{4\theta-1}{3} \right) \frac{2}{3} = \frac{4(1-\theta)}{9n} \quad X$$

$$- \frac{4(1-\theta)}{3n} - \frac{2}{n} \left(\frac{4\theta-1}{3} \right) \frac{4}{3} = - \frac{4(1+5\theta)}{9n} \quad XI$$

$$- \frac{4(1-\theta)}{3n} - \frac{2}{n} \left(\frac{4\theta-1}{3} \right) \frac{4}{3} = - \frac{4(1+5\theta)}{9n} \quad XII$$

$$\frac{4(2+\theta)}{3n} - \frac{2}{n} \left(\frac{4\theta-1}{3} \right) \frac{2}{3} = \frac{4(9-9\theta)}{9n}$$

Since n does not appear explicitly $\frac{\partial r}{\partial n} = 0$. Substituting the values in equations X to XIII in equation V, squaring and multiplying by the expected frequencies

$$\frac{1}{n} V(r) = \left(\frac{4}{9n}\right) \frac{1}{4} \{ (1-\theta)^2 (2+\theta) + 2(1-\theta)(1+5\theta)^2 + 81\theta(1-\theta)^2 \}$$

$$\text{or} \quad V(r) = \frac{16}{81n} (1-\theta) (1+25\theta-8\theta^2)$$

$$\text{Since} \quad V(\theta) = \frac{9}{16} V(r)$$

$$V(\theta) = \frac{(1-\theta) (1+25\theta-8\theta^2)}{9n}$$

The variance of $\sqrt{\theta}$ or p , will then be

$$\frac{V(\theta)}{4\theta} \quad \text{or} \quad \frac{(1-\theta) (1+25\theta-8\theta^2)}{36n\theta} \quad \text{XIV}$$

and the standard error of p may then be expressed conveniently

$$\text{as} \quad \sqrt{\frac{(1-p^2) (1+25p^2-8p^4)}{36np^2}} \quad \text{XV}$$

Fisher (2) has also shown that the method of maximum likelihood, in the theory of large samples, will in all cases give a standard error as small as possible. The efficiency of the correlation method can then be tested by dividing the variance for the maximum likelihood method (3, p. 250) by that of the correlation method. This quantity,

$$\frac{2\theta(1-\theta)(2+\theta)}{(1+2\theta)n} + \frac{(1-\theta)(1+25\theta-8\theta^2)}{9n} = \frac{18\theta(2+\theta)}{(1+2\theta)(1+25\theta-8\theta^2)} \quad \text{XVI}$$

will then give a measure of the efficiency of the correlation method for all values of $\theta (= p^2)$. The result is shown graphically in Fig. 1, as well as the apparent efficiency given by the sampling error deduced on the assumption of a normal correlation surface as given in equation IV.

It is seen readily that the curve for the actual efficiency of the correlation method calculated from the correct formula, equation XV, does not exceed 100 per cent. for any possible values of p , from 0 to 1, in accordance with the general theory. The correlation method is fairly efficient in the coupling phase and for loose linkage in repulsion. For close linkage in repulsion it is not efficient. Since there are other formulae (3), such as the maximum likelihood method and the product ratio method, which are efficient for all values of p , it would seem preferable to use these formulae in most cases.

The error formula based on the incorrect method of treating the fourfold table of phenotypic frequencies as a normal frequency surface gives more than 100 per cent. efficiency in the coupling phase, which is obviously impossible. It is only for the value of $p = .50$ that this formula is cor

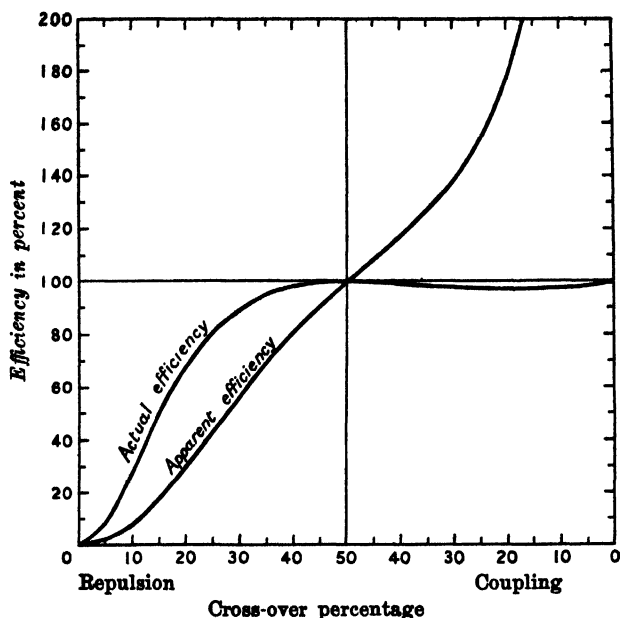


FIG. 1

Graph showing actual efficiency of correlation method for estimating linkage intensities and its apparent efficiency when the standard error of the correlation is incorrectly calculated.

For close linkage in coupling the two recombination classes, b and c , may be very small. In fact, recombinations in b or c may be entirely lacking. Under such conditions the theory of large samples breaks down and some of the efficient statistics fail. For close linkage b and c will be small numbers, either of which may be zero, while a and d will be approximately $\frac{1}{2}$ and $\frac{1}{2}$ of the sample respectively.

If b and c are small compared with a and d , then r may be expressed as

$$\left(1 - \frac{bc}{ad}\right) \left(1 - \frac{b}{a} + \dots\right) \left(1 - \frac{c}{d} + \dots\right) \left(1 - \frac{c}{d} + \dots\right) \left(1 - \frac{b}{d} + \dots\right) \\ = 1 - \frac{1}{2} (b+c) \left(\frac{1}{a} + \frac{1}{d}\right),$$

neglecting squares and products of $\frac{b}{a}$, etc.

Then

$$p = \frac{1}{2} \sqrt{4 - 3/2 (b+c) \left(\frac{1}{a} + \frac{1}{d}\right)} \\ = 1 - 3/16 (b+c) \left(\frac{1}{a} + \frac{1}{d}\right)$$

to the same approximation. Putting the limiting values $a = \frac{1}{2}n$ and $d = \frac{1}{2}n$ in the expression, $p = 1 - \frac{b+c}{n}$. This is the same result as is given for this case by Emerson's method and by maximum likelihood.

Contrast the product method

$$\frac{(1-p')^2}{p'(2+p')} = \frac{bc}{ad}$$

When b is 0 and c is not, cross-overs must have occurred, and yet $1-p$ is estimated to be exactly zero, which is manifestly wrong. It would seem, therefore, that the product ratio method should not be used when the observed numbers in the b and c classes are very small, i.e., less than a total of about ten. For very close linkages in coupling when b and c are small the maximum likelihood, Emerson's or the correlation method would be preferable to the product ratio. The maximum likelihood method might claim a theoretical advantage since it is efficient for all values of p .

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ON THE OCCURRENCE OF *TRICHOCORIXA* KIRKALDY
(CORIXIDAE, HEMIPTERA-HETEROPTERA)
IN SALT WATER AND ITS ZOO-GEO-
GRAPHICAL SIGNIFICANCE

IN spite of the considerable literature on the mechanisms by which animals and plants might be dispersed, too little attention has been given to the physiological feasibility of the methods of distribution invoked to explain wide and discontinuous ranges. The following records appear to indicate a case where dispersal by ocean currents is within the limits of physiological possibility and may be legitimately offered as an explanation of a very wide and remarkable distribution.

Trichocorixa Kirkaldy is a genus of water-boatmen of wide distribution in South, Central, and southern North America. Several forms are recorded from the West Indies and the genus would appear to have a distribution typical of many groups of Central or South American origin were it not for a single species which ranges right across the Pacific from California to China. The following notes deal with this form, *T. wallengreni* (Stål), and its close Eastern ally, *T. verticalis* (Fieb.).

During fisheries investigations in Delaware Bay in 1929 Mr. Albert E. Parr, curator of the Bingham Oceanographic Foundation, Yale University, obtained two living ♂ specimens of *Trichocorixa verticalis* (Fieb.) associated with typical marine planktonic organisms in tow-nettings taken at stations 48, north of Brandywine Shoal, salinity 24.90 per mille (June 18, 1929) and 63, salinity 29.34 per mille (June 18, 1929). Although drowned flies and other insects were frequently met with in the surface plankton of this region, no specimens of living and apparently healthy insects other than these two corixids were obtained. *T. verticalis* occurs commonly in ponds near the sea in Cape May County, N. J., and is recorded from Connecticut, Pennsylvania, Georgia and the West Indian Islands of Cuba and St. Thomas (Lundblad, 1929).

Mr. Richard M. Bond, Bishop Museum fellow of Yale University, has forwarded for determination a number of specimens of the closely allied *Trichocorixa wallengreni* (Stål) taken in "strong brine from salt works at Elkhorn Slough," Monterey County, Cal. (10th November, 1930). Both sexes as well as immature individuals occurred in this locality, which otherwise is

inhabited only by the typical halobionts *Dunaniella*, *Artemia* and *Ephedra*. *Trichocorixa wallengreni* was originally described from California, but recently Lundblad (1929b) has shown that *Corixa blackburni* Buch.-White from Hawaii is synonymous and has also recorded the species from Shanghai. This transpacific distribution is probably unique among waterbugs; the Hawaiian records strongly suggest that it is to be explained by dispersal across the Pacific Ocean, rather than by an Alaskan-Siberian land-bridge. *T. wallengreni* or its eggs might possibly be transported in damp salt, but the species has clearly been established for some time in Hawaii (*C. blackburni* was described by Buchanan-White in 1877) and a natural method of dispersal seems more probable. Since it is clear that the species can stand salinities considerably above those of the sea it is not inconceivable that specimens might travel by the Northern Equatorial Current from California to Hawaii, and from Hawaii to China. Insects of this family being less dense than water when surrounded by their air bubble, this method of distribution would involve a minimum of effort.¹

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¹ Since the above was written, Dr. H. B. Hungerford kindly informs me that he has specimens of *Trichocorixa* from the Galapagos Islands and that he has frequently received specimens of the genus "from saline waters. The exact salinity of the water, however, has never been available."

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- 47, 49, 51, 52, 60, 62) or less commonly, as in some species of *Palpomyia*, distinctly separated (figs. 53, 65) although they may be connected at base (fig. 66). Larvae actively swimming eel-like forms, without prolegs. Head narrow, well sclerotized; mouth parts reduced; pharyngeal skeleton with three combs; principal comb of angulus divided. Abdominal segments 3 to 7 of pupa similarly bristled; anal segment with bristles on bristle tubercle. Pupa free from larval exuviae; respiratory trumpet clavate, with numerous spiracles (*PALPOMYIA-BEZZIA* GROUP) 29
29. Anal lobe of wing large, produced; wing broad, especially at base (figs. 36, 43); costa usually more or less produced beyond the end of the posterior branch of the radius; either one or two anterior radial cells 30
30. Anal lobe obtuse angled, not produced; wing usually of moderate width 31
30. Posterior branch of media elbowed in female; a single very narrow anterior radial cell (fig. 43); costa elongate, extending to tip of wing. Femora unarmed; fourth tarsal segment not bilobed, subcylindrical; fifth segment unarmed; claws small and equal *Stenoxenus*
- Posterior branch of media not elbowed in the female; one or two anterior radial cells (fig. 36); microtrichia present; medial fork broadly sessile. Anterior margin of thorax somewhat jutting over the head but not pointed. Femora unarmed, fourth tarsal segment cordate or bell-shaped, broader than long; fifth segment with strong, blunt spines (batonnets) below *Jenkinshelea*
31. With two anterior radial cells (fig. 39) 32
- With but one anterior radial cell (fig. 40) 40
32. Costa produced beyond the apex of the posterior branch of the radius (fig. 39); medial fork just sessile. Fourth tarsal segment cordate; fifth segment of fore legs swollen in both sexes as in *Clinoshelea*; claws in the female all equal and barbed *Neurohelea*
33. Costa not produced beyond tip of posterior branch of radius 33
33. Femora of fore legs armed and greatly swollen, the corresponding tibiae arched (fig. 24); hind legs of female very long, with a single long, basally barbed claw (fig. 29); claws of male small and equal; first and second pairs of the female equal and barbed. Apical third of hind femur of both sexes distinctly enlarged; fourth tarsal segments on all feet of the male and the first two pairs of the female, cordate. Antenna of male plumose. Wings of the North American species fasciate (fig. 38) *Heteromyia*
- Fore femora not swollen, or if moderately so, fore tibia not arched 34
34. Claws of middle and hind legs of the female unequal and simple, or a single claw with a basal barb; fifth segment of fore tarsi inflated (fig. 27) and unarmed on all legs; fourth tarsal segment on four posterior legs deeply bilobed, each lobe terminating in a strong spine. Venation as in *Heteromyia* *Clinoshelea*
- Claws of hind legs of female equal, or if unequal then each with a basal barb 35
35. Femora and tibiae all armed with spines, some rather long, scattered irregularly; tarsi with short fourth segment which is not clearly cordate or bilobed; fifth segment unarmed; claws barbed, equal or unequal. Second radial cell much longer than the first. South America . . . *Echinohelea*
- Femora either unarmed, or if armed the spines are short and stout 36
36. Claws unequal in the female, at least on middle and hind feet, and all barbed; fourth tarsal segment not cordate; fifth armed below with straight, somewhat blunt, black spines (batonnets); femora with or without spines on the under side. Venation as in *Heteromyia*. Minute notal spine present as in *Palpomyia* in the North American species *D. argentalis* (Loew) and *D. annulicornis* (Malloch) *Dicrohelea*
- Claws of female all equal 37
37. Claws of female without basal barb and as long or nearly as long as the last tarsal segment; fourth segment but little longer than broad, cordate or nearly so; fifth segment of female armed with stout blunt spines (batonnets) on the under side. Oriental and South American species. The North American species assigned here by Kieffer belong elsewhere. *Homoshelea*
- Claws of female all equal and barbed, or, if not barbed, stout blunt spines (batonnets) are lacking in the female on the under side of the fifth tarsal

- segment, although stout, sharp, more or less curved bristles may be present. 38
38. Femora without strong spines below; fifth tarsal segment in the female armed with stout, blunt, black spines below (batonnets). Venation as in *Heteromyia*. Scutum without short minute, spine or tubercle anteriorly; abdomen of female without eversible glands. **Johannsenomyia**
- Femora armed with short stout black spines below. 39
39. Tarsi with fourth segment short, subcylindrical. Stout, robust species. Eyes nearly contiguous. Scutum in most cases without minute, anteriorly directed spine. Abdomen without eversible glands in the female. Fifth tarsal segment in the female with stout, black, blunt spines (batonnets) on the underside. Male antennae either with or without plume, **Sphaeromias**
- Fourth tarsal segment cordate; fifth segment without stout, blunt spines below, but stout, sharp, somewhat curved bristles may be present. Scutum with a minute, pointed spine or tubercle anteriorly, sometimes apparently lacking. Abdomen of female with pairs of eversible glands in many species. Forceps of hypopygium with simple terminal segment; the inner caudad projecting processes either separate (fig. 53), or partly (fig. 66), or completely fused (fig. 47). Fore femora either slender or moderately swollen, with one or more short, stout spines on the lower side; middle and hind pairs slender, with or without stout spines below. Tarsal claws of moderate size, equal, with or without basal barbs. Wings rather narrow, with fine microtrichia but without macrotrichia. Venation as in *Heteromyia*, the costa extending to two-thirds the wing length or more, not produced beyond the end of the posterior branch of the radius; second anterior radial cell much longer than the first; median fork broadly sessile; anal lobe not well marked, alula absent, the short piece of wing margin between the anal lobe and the squama without fringe. **Palpomyia**
40. Wing without macrotrichia. 41
- Wing with macrotrichia (fig. 42); venation as in *Dicrobezzia* (fig. 41) or in *Probezzia* (fig. 40). Fourth tarsal segment cordate; empodium absent. In the genotype, *L. pilipennis*, from north Sweden, the fore femora are armed, the claws are simple, the fifth tarsal segment unarmed in the female, and the posterior branch of the radius ends at about two-thirds the wing length as in *Probezzia*. In the New York species, *L. unica*, the femora are unarmed, the fifth tarsal segment with stout, blunt spines below (fig. 28), the claws equal and barbed, the posterior branch of the radius elongate, disc of the scutum with short, erect bristles as in *Dicrobezzia* (fig. 23). The presence of the macrotrichia on the apical half of the wing being probably of less significance than the other characters cited the two species are not closely related. **Lasiobezzia**
41. Fourth segment of all tarsi bilobed or cordate. 42
- Fourth segment of tarsi subcylindrical; claws of female equal and with basal barb. 45
42. Mesonotum but slightly convex, with a prominent, anteriorly, and somewhat upward directed tubercle on the anterior margin (fig. 22). Abdomen elongate. Antenna of the female with short, stout, sparsely distributed hairs (fig. 20). Tarsal segments three to five very short, the fourth segment bilobed or cordate. In the genotype *C. fallax* K. from Paraguay the fifth tarsal segment of the fore legs is inflated, which is not the case in *C. flavida* Joh. from British Guiana. **Ceratobezzia**
- Mesonotum without large, stout spine on the anterior margin. 43
43. Costa of the wing and second radial cell of the female very long, reaching nearly to the wing tip (fig. 41). Dorsum of the thorax with short, sparsely distributed, erect bristles in addition to the decumbent hairs (fig. 23). Femora unarmed; fifth tarsal segment of the female with strong, blunt, black spines (batonnets) below. **Dicrobezzia**
- Costa not prolonged. Fifth tarsal segment of the female unarmed. 44
44. At least the fore femora spinose beneath; fifth tarsal segment of fore feet not inflated; claws of female equal on all legs. Venation as in *Probezzia* (fig. 40), the fork of the media narrowly sessile, the contact often punctiform. Scutum without trace of minute anterior tubercle. **Bezzia**